PB83-149377

Factors Influencing Metal Accumulation by Algae

Syracuse Univ., NY

Prepared for

Industrial Environmental Research Lab.
Cincinnati, OH

Dec 82

U.S. Department of Commerce
National Technical Information Service
NTIS

EPA-600/2-82-100
December 1982

PB83-149377

FACTORS INFLUENCING METAL ACCUMULATION BY ALGAE

by

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Grant No. R804734

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SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/2-82-100

2.

3. RECIPIENT'S ACCESSION NO.
PB83 149377

4. TITLE AND SUBTITLE
FACTORS INFLUENCING METAL ACCUMULATION BY ALGAE

5. REPORT DATE
December 1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
J.Charles Jennett, J.E. Smith and J.M. Hassett

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Syracuse University
149 Hinds Hall
Syracuse, NY 13210

10. PROGRAM ELEMENT NO.
1BB610

11. CONTRACT/GRANT NO.
R804734

12. SPONSORING AGENCY NAME AND ADDRESS
Industrial Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

13. TYPE OF REPORT AND PERIOD COVERED
Final Report, 9/76-1/79

14. SPONSORING AGENCY CODE
EPA/600/12

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Shallow beds of algae (algal meanders) have proved to be highly effective at removing heavy metals and organometallics from lead-zinc mine and mill wastes. A research program was initiated (1) to determine conditions under which algae were most effective at concentrating significant quantities of As, Cd, Cu, Hg, Ni, Pb, or Zn, and (2) to apply the meander technology to new types of wastewater-metal problems.

Studies were performed on 20 species of algae to determine interactions of experimental variables affecting metal adsorption; species and strains of algae; type, form, and concentrations of metal; pH; culture age; micronutrient composition of culture medium; exposure to metal; and light intensity and exposure period. These numerous variables were studies by means of a rapid analytical technique, the Titertek™ supernatant collection system, to determine the adsorption of metal radionuclides.

Neither Zn nor As were bound significantly at any pH. Experimental and literature data indicate that algae remove certain metals economically from a variety of waters and that the meander system can be used to recover these metals for processing.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
Release to Public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
133

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLI

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FOREWORD

When energy and material resources are extracted, processed, converted, and used, the related pollutional impacts on our environment and even on our health often require that new and increasingly more efficient pollution control methods be used. The Industrial Environmental Research Laboratory-Cincinnati (IERL-Ci) assists in developing and demonstrating new and improved methodologies that will meet these needs both efficiently and economically.

This report describes a detailed laboratory study of factors influencing heavy metal accumulation by 20 species of algae. Laboratory conditions under which particular algae were most effective at concentrating significant quantities of arsenic, cadmium, copper, mercury, nickel, lead or zinc were determined. These data can be utilized further to evaluate the feasibility of using algal meanders to remove a variety of heavy metals from industrial wastewaters. The Energy Pollution Control Division is to be contacted for further information.

David G. Stephan
Industrial Environmental Research Laboratory
Cincinnati

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 281)
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ABSTRACT

Shallow beds of algae (algal meanders) have proved to be highly effective at removing heavy metals and organometallics from lead-zinc mine and mill wastes. A lab-scale research program was initiated to determine conditions under which algae were most effective at concentrating significant quantities of As, Cd, Cu, Hg, Ni, Pb, or Zn, for the purpose of potential application of the meander technology to new types of wastewater-metal problems.

Studies were performed on 20 species of algae to determine interactions of experimental variables affecting metal adsorption, species and strains of algae, type, form, and concentrations of metal, pH, culture age, micronutrient composition of culture medium, exposure to metal, and light intensity and exposure period. These numerous variables were studied by means of a rapid analytical technique, the Titertek^{TM} supernatant collection system, to determine the adsorption of metal radionuclides.

Metal removal was observed to be fast (3 hrs), young growing cultures were seen to be most effective, and concentration factors $> 1 \times 10^4$ were observed. The pH had little effect on accumulation of metals except lead. Heavy metals could be stripped from algal mats with 0.01 M EDTA or 0.1 N HNO_3. Ca and Mg were not effective competitors for the binding sites of Hg, Pb and Cd. Neither Zn nor As were bound significantly at any pH. Experimental and literature data indicate that algae remove certain metals economically from a variety of waters and that the meander system can be used to recover these metals for processing.

This report is submitted in fulfillment of Grant No. R804734 by Syracuse University under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period from September 13, 1976 through January 1, 1979 and work was completed as of June 30, 1979.

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SECTION I

INTRODUCTION

Effluents from secondary lead smelters are a major source of heavy metals in the aquatic environment. These effluents are frequently low in pH and contain a wide range of heavy metals. The largest single heavy metal constituent is, of course, lead. Relatively large amounts of zinc, cadmium, arsenic, copper, and other toxic cations may also be found, along with a wide variety of anions often associated with these metals (chloride, sulfate, nitrate and chromate for example). Lead smelter effluents also contain toxic trace organics generated when batteries and other materials are crushed and the heavy metals are removed from their plastic encasements. The fact that heavy metals are dangerous in the aquatic environment has been well documented in the literature. The problem is a major one and prevalent throughout the United States and the rest of the world.

One possible solution is to apply the type of algal-meander treatment system currently in use for treating mine and mine-mill wastes at one site in the new lead belt region in southeast Missouri--the world's largest lead mining region (Jennett and Wixson 1975a). A detailed laboratory study of the factors influencing heavy metal accumulation by algae is the subject of this investigation and an attempt has been made to evaluate the feasibility of using algal-meanders to remove a variety of heavy metals.

DESCRIPTION OF THE MEANDER PROCESS

In the Missouri algal-meander system, mine and mill wastes were treated in a standard tailings pond followed by a series of shallow meanders in which the growth of algae is encouraged. These algae grow, utilize the waste millings reagents and trap heavy metals, both particulate and dissolved, on their surface. When the algal mat breaks loose, it is then trapped in a settling pond at the end of the meander system. The pond is equipped with baffled weirs which prevent algal overflow into the receiving stream. Based on total heavy metals removed, the system is more than 99 percent effective. Dissolved heavy metals are also removed to levels well below U.S. Public Health Service drinking water standards. Work by N.L. Gale at the University of Missouri-Rolla (Wixson and Jennett 1974) has shown also that lead in its highly soluble acetate form is removed effectively by the algae.

The process has been shown to be an adsorptive one and temperature is not a major factor in removal (Jennett and Wixson 1975b). The algae do not become a solid waste problem (Jennett and Wixson 1975a, b; Wixson and Jennett

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1974, Ryck and Whitely 1974). Solids do build up in the final sedimentation pond for 1 to 2 years, but then this buildup stops or slows down in a manner analogous to sewage lagoons. Furthermore, the algae with their coating of heavy metals become relatively dense and settle well. No problems have been reported with floating material. The leaching of heavy metals from the entrapment through the soil to the groundwater table must be checked at each individual site but most normal soils (except sand) have such high cation exchange capacities that such leaching is a highly unlikely event (Linneman 1975).

To date the algal-meander system has been used to treat only one waste, a combined lead-zinc mine and mill waste in the new lead belt mining region. Since 1971, this region has been the site of a large interdisciplinary investigation, supported by the National Science Foundation, of environmental pollution by lead and other heavy metals. Wixson and Jennett in 1974 confirmed that below the AMAX-Homestake Lead Tollers, Inc. mining mills, severe stream degradation and heavy metal buildup was occurring. During the course of their study, the meandering system was built to a full operating scale and as a result the stream was returned to essentially pre-mining conditions (Jennett and Wixson 1975a,b; Ryck and Whitely 1974). The history of this treatment system and the quality of the water in receiving streams has been well documented for lead and zinc (Jennett and Wixson 1975a,b; Wixson and Jennett 1974). This technology, however, has not been applied to wastes containing other toxic metals. This has been largely due to the lack of information on the system's range of application and the newness of the technology. The ability of algae to concentrate heavy metals from their aqueous environment has been reported by radioecologists examining debris from nuclear tests (Polikarpov 1966), chemical geologists attempting to explain low-grade deposits of metal (Ferguson and Bubella 1974), and ecologists concerned with the self-purification of streams below mining operations (Trollope and Evans 1976). While these observations are clearly valuable, a more detailed knowledge of the interaction between algae and heavy metals could aid in understanding these processes. A systematic study of these interactions has been difficult because a wide range of variables affects metal accumulation by algae. These variables include the length of the exposure period, the type of metal, oxidation states, pH, salinity, and presence of organic pollutants. To understand the factors influencing metal accumulation by algae in the meander system, laboratory studies were initiated with pure cultures of representative species from other laboratories and from the algal culture collection at the University of Texas (Starr 1964).

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SECTION 2

CONCLUSIONS

The ability of representative algae to concentrate large quantities of specific heavy metals was demonstrated by adapting the Titertek^{TM} supernatant collection system (Flow Laboratories, Rockville, Maryland) for assaying radionuclides of heavy metals. This technique permitted the simultaneous study of several factors affecting heavy metal accumulation and provided a sufficient number of replicates for statistical analysis.

Calculation of concentration factors for heavy metals revealed significant differences between species of the same genus and between strains of the same species (i.e. Nostoc). Some species regularly had concentration factors of 1×10^4. In general, young cultures exhibited very much higher concentration factors than old cultures. Most of the metal removed by algae can be removed by a rinse with 0.01 M ethylenediaminetetraacetic acid (EDTA) or 0.1 N nitric acid (HNO_3). This information suggests that surface adsorption sites are the principal repository for the metallic ions or particles.

The ability of algae to remove metals is generally in this order: mercury > lead > cadmium. Neither zinc nor arsenic was removed significantly at any pH during a 3-hour exposure. The presence of chelating agents in the medium (such as EDTA) inhibited the removal of the heavy metals. Calcium and magnesium were not effective competitors for the binding sites of mercury, lead, and cadmium. There was remarkably little effect of pH on metal accumulation in the range of pH 5 to 8 when young cells were employed. Lead was the principal exception because it was removed more efficiently at pH 4 to 5 than at other pH values.

The removals of lead and mercury from nutrient solution by algae are found to be rapid phenomena, usually accomplished in three hours or less at room temperature. If the cells were placed in buffer after the initial adsorption of lead, a limited, slow release of lead occurred for a few hours, perhaps while some surface polymer-lead complexes were released from dead or dying cells. Mercury was removed from solution in nearly identical fashion by two strains of Nostoc and showed little evidence of being released. In the control wells without algae there were reproducible losses of ^{203}Hg that occurred within the first 24 hours at pH 6. This may be related to reports in the literature claiming volatilization of mercury by algae.

Chlamydomonas (a green flagellated form) proved to be dramatically superior to all other species in its ability to remove lead. Concentration factors for the organism of 1.9×10^4 were noted. This organism showed essentially the same concentration factor for lead in the pH range of 4 to 9.

In other experiments, Ulothrix and Chlorella had concentration factors for cadmium greater than 10^4.

The empirical successes of the algal-meander system for removing lead from surface waters can be explained in part by the concentration and sequestering of lead and cadmium on the surface of algal cells. Very little heavy metal was leached from these cells at pH 5 to 9 in the presence of divalent cations and other inorganic constituents of algal growth media.

SECTION 3

RECOMMENDATIONS

The remarkable ability of selected algae to concentrate heavy metals on their cell surfaces suggests that the use of intensive algal culture may be a very practical way to reclaim wastewater and/or recover useful quantities of metals. Several directions for future research and development are clearly indicated before this potential can be realized:

A. The glycocalyx--i.e., the mucopolysaccharide and protein capsule surrounding all algae must be isolated from metal-avid species and studied for its biochemical structure, exchange capacities, and biphasic physical properties. With these data in hand, it is practical to search for organisms with glycocalyces having desirable properties for the adsorption of metals.

B. If algal beds are to be maintained on a continuing basis, it will be necessary to follow the reproductive capacity of the organism(s) in the presence of excessive levels of metal. In fact, it may be necessary to devise schedules for metal adsorption that are followed by regeneration at low metal levels.

The present meander technology depends entirely on the presence of naturally occurring wild algae, probably growing as a mixed flora. Since some of the species may have concentration factors in excess of 1×10^4 under certain conditions, and others may not concentrate metal at all, it appears profitable to encourage the former by selective algal farming. Simple enrichment culture techniques are feasible for the meander manager.

C. A screening operation should be devised with the objective of finding natural or induced mutants that a) have accumulation coefficients one or more orders or magnitude greater than wild types, b) exhibit greater selectivity for a particular metal ion than the wild types, and c) tend to remain in their benthic form rather than in their free floating form. The blue-green algae may be the better choice for this work because they are usually more metal-avid than green algae, and because they are haploid. The latter characteristic makes it easier to induce mutations and to select for variants in blue-green algae diploid organisms.

D. Almost no data are to be found concerning the maintenance of beds of benthic algae on a continuous basis. Experiments are needed to learn the long-term consequences of heavy metal accumulation in such beds, to monitor detention and release of metals during chronic exposures, and to study the detachment of benthic cells coated with heavy metals.

E. Methods must be devised for systematically discharging metals, or for otherwise recovering them, from meander beds. Simultaneously, it must be determined whether or not it is feasible to recycle the algal cells as inoculum or feedstock after they have been stripped of metal by chemical washing.

F. Investigation of practical questions regarding the suitability of metal-stripped secondary sewage effluent for agricultural purposes must be examined. Conversely, the efficacy of sewage as a dependable nutrient source for algae has not been established. Certainly the presence of oil, cyanates, solvents, etc., could be expected to interfere with the productive synthesis of the glycocalyx adsorptive surface.

G. The effects of light, temperature, and salt concentration have not been evaluated on a continuously operating meander. Quantitative data have not been collected on the optimum output of glycocalyx because the factors which stimulate it are essentially unknown.

H. It should be determined whether or not an appropriate substitute can be found for the algal cells in the meander (aggregates or other surfaces coated with semi-purified glycocalyx, for example). This search might provide a renewable and/or strippable surface that would eliminate some of the vagaries associated with the maintenance of open algal cultures.

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SECTION 4

LITERATURE REVIEW

INTRODUCTION

The literature contains numerous studies concerning the effects of heavy metals on algae. The topics studied include toxic and mutagenic effects, suppression of growth, effect on photosynthetic rates, assessment of certain algae as indicators of metallic pollution, development of resistance to heavy metals, and concentration of heavy metals through food chains and biotransformation studies. The review presented here will concern itself with these studies as well as the phenomenon of algal concentration of heavy metals, and the effects of heavy metals on the morphology of algae.

MORPHOLOGY

Gertz and Suffet (1977) state that the first structure of an algal cell encountered by a heavy metal is the algal cell wall. Though this is true in many cases, the great morphological diversity of algae makes such a statement overly simple. Table I summarizes the various categories of cell coverings found in some algal groups. These categories include naked membrane , stage, normal, pellicle, silica frustule, silica scales, sheath or capsule.

In this table, membrane refers to the plasmalemma or cell membrane, usually 7 to 8 nm thick with an external "fuzz" of proteinaceous material. Stage is the term for zoospores or gametes that are reproductive structures present in the life cycles of some algae. Normal refers to organisms possessing a cell membrane for their entire life cycle. Pellicle is a structure with an outer plasma membrane, under which lie flat strips of proteinaceous material, often with muciferous bodies at various points on the pellicle. A silica frustule is a complex silicaceous structure found exclusively in diatoms. The plasmalemma remains outside while the frustule is forming; after formation, the outer membrane is abandoned, and a new plasmalemma is formed inside the cell. Silica scales refer to silacaceous coverings found outside the cell membrane (Dodge 1973).

The sheath or capsule is the covering external to the cell wall found in the blue-green algae. It is either a thick and solid structure or a thin and dissolving slime layer (Drews, 1973). These sheaths are polysaccharide in nature (Fuhs 1973). In the blue-green algae, the cell walls are multilayered and composed of murein (the rigid, formative component of the cell wall) carbohydrates (mannose, galactose, xylose, and glucose polymers), amino acids, fatty acids, and possibly murein-associated proteins (Drews 1973).

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Other components found include rhamnose, arabinose, ribose, and glucuronic acid (Fogg, et al., 1973).

In algae other than the blue-greens, the cell wall, when present, is either incomplete or complete. In the former case, the portion of the cell wall present (called a lorica) is constructed of microfibrils thought to be cellulosic in nature. On the other hand, algae with complete cell walls show a wide variety of structures. One red alga studied had a cell wall constructed of microfibrils of cellulose. The yellow-green algae show randomly arranged cellulosic microfibrils (Dodge 1973).

Green algae in general show cell walls made up mostly of cellulose walls which consist mainly of cellulose microfibrils. An example of this is Cladophora. Sometimes the wall is an outer protein-rich cuticular region with several alternating microfibrillar and amorphous layers. Ulva exhibits a less distinct organization in its cell wall with a larger amount of incorporated amorphous materials. Spirogyra has an outer layer of mucilaginous materials, and Chlorella is coated with a layer of sporopollenin. In Chlamydomonas, the wall is composed, at least in part, of proteinaceous materials (including glycoproteins). Some green algae have cell walls based on xylan and mannan rather than cellulose (Dodge 1973).

OBSERVATIONS CONCERNING METAL CONCENTRATION BY ALGAE

General

The removal and concentration of heavy metals from their aqueous environment by algae was first studied in detail in the 1950's and 1960's as part of the problem of the release of nuclear debris from weapons testing and from reactor cooling water effluent. Scientists took advantage of the released isotopes, as well as newly developed analytical techniques, to study pathways of various elements in the environment. One early result of this work was the observation that certain isotopes were concentrated by biota to a much greater extent than the concentration of that isotope in water. From these observations came the concept of the concentration factor (CF) such that

$$CF = \frac{C}{C^1}$$

where C and C^1 are respectively the concentration of the radionuclide in the aquatic organism and in the aqueous medium. The concept is also applicable to stable isotopes (Polikarpov, 1966).

This rather new branch of science (radioecology) was necessarily limited to isotopes with long half-lives that were produced under conditions mentioned above. Furthermore, field observations were limited to areas close to test sites and/or reactors and laboratory work emphasized the accumulation of fission products. The early data were, therefore, rich in information on isotopes of cerium, yttrium, zirconium, niobium, molybdenun, iodine, strontium, germanium, and praseodymium, as well as the induced radioisotopes of zinc, phosphorous, chromium, sulfur, manganese, iron, and cobalt (Polikarpov, 1966).

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TABLE 1. SUMMARY OF CELL COVERINGS FOUND IN SOME ALGAL GROUPS

Algal Type	Naked Membrane Stage Only	Naked Membrane Normal	Pellicle	Silica Frustule	Silica Scales	Sheath or Capsule	Cell Wall Incomplete (Lorica)	Cell Wall Complete	Reference
Bacillariophytes (Diatoms)	+			‡					Dodge (1973)
Chlorophytes (Green algae)	+	+						‡	"
Chrysophytes (Golden algae)		+			‡		+		"
Cyanophytes (Blue-green algae)		+				‡		‡	Drews (1973)
Euglenophytes (Brown algae)			‡						Dodge (1973)
Phaeophytes (Brown algae)	+							‡	"
Rhodophytes (Red algae)		+						‡	"
Xanthophytes (Yellow-green algae)	+							‡	"

After Dodge (1973)

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More recently, however, emphasis has shifted to the study of elements of industrial and ecological importance--principally arsenic, cadmium, copper, lead, mercury, nickel, and zinc.

Data available from the literature have been assembled into Tables A-1 to A-7, which describe the information available about a specific metal. The data were produced by the following types of observation: radioecological (field observations involving measurements of radioisotope contaminants), ecological (field studies involving stable isotopes of the metal in question), radioisotope (laboratory studies involving radionuclides as tracers), and stable isotope (small scale laboratory experiments using stable isotopes).

Data presented in Tables A-1 to A-7 require some further explanation. Biomass was determined in some cases on a wet (fresh) weight basis and in other cases on a dry weight basis. Data from Booth and Knauer (1972) indicate that, for algae, wet weight is greater than dry weight by a factor of approximately 57, a point to be considered when comparisons are made from the tables.

A third point to consider in data interpretation has to do with epiphytic organisms. One recent study (Patrick and Loutit, 1977) noted that a biological slime consisting of protozoans, diatoms, and bacteria of the genus Sphaerotilus coated the macrophyte Alisma plantago-aquatica growing downstream from an industrial discharge. Removing these epiphytes from leaf surfaces removed some iron and lead, 15-50% of the chromium, 30-35% of the copper and 10-50% of the zinc. The authors attribute most of the reduction in adsorbed metals to the removal of the bacteria.

Some interesting observations are presented about metal concentration by algae in Tables A-1 to A-7. First, all the metals are concentrated to some degree. Second, there are differences in the degree of concentration among metals for a given alga. For example, the marine brown alga Fucus vesiculosis has a cadmium concentration factor (CF) of 2.7×10^3, copper CFs of 2.5×10^3 to 2.7×10^4, a lead CF of 2.4×10^3, nickel CFs of 2.8×10^3 to 6.8×10^3 and zinc CFs of 4.2×10^2 to 6.4×10^4, all values calculated on a dry weight basis. Using the maximum CF reported for each metal gives an order of Zn > Cu > Ni > Cd > Pb. If the minimum CF is chosen for each metal, however, the order changes to Ni > Cd > Cu > Pb > Zn. Some of the reasons for the variations of CFs for a metal could include differences in ages among specimens collected, seasonal variations, temperature differences.

A wide range of CFs was observed for particular metals among several different algae. For example, Zygnema had a nickel CF of 6×10^3 while Microspora, collected from the same stream, had a nickel CF of only 36. Both are filamentous green algae. It therefore appears that some algae scavenge metals more efficiently than others and it is not due to bacterial epiphytes growing on their surfaces.

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OBSERVATIONS ON THE INTERACTIONS BETWEEN METALS AND ALGAE

Arsenic

Radioecological studies of arsenic and algae are made difficult by the fact that the most important radionuclide of arsenic, the neutron-induced ^{76}As, has a half-life of only 26.8 hours (Polikarpov 1966). Therefore, little work has been done on this subject.

Arsenic is found in water in various anionic forms, depending on pH and Eh: H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$, AsO_3^{3-}, $H_2AsO_4^-$, and $HAsO_4^{2-}$ (Ferguson and Gavis 1972). Other forms are possible under extreme conditions as a result of industrial discharges (National Research Council, 1977a).

Lunde (1972) studied the accumulation of arsenic organic compounds in algae derived from the inorganic arsenic present in culture media. The five species studied, with the concentration factor being the ratio between organic-bound ^{76}As in either a lipid or an aqueous extract and the inorganic ^{76}As in the medium, had CFs of 2.4×10^2 to 2.9×10^3. The concentrations were determined by neutron activation analysis. The lipid phase invariably contained more arsenic than the aqueous extract; *Phaeodactylum tricornutum* had the highest concentrations of arsenic.

The same worker (Lunde, 1970) measured the arsenic contents of known algae at various points along the European coast. Using neutron activation analysis, he determined that brown algae of the family Laminariaceae had higher affinities for arsenic than members of the family Fucaceae. Other experiments done on a laboratory scale with labelled sodium arsenite showed that the test algae achieved high concentrations in as short a time as 2 hours. A rinse of dilute hydrochloric acid removed the arsenic, indicating surface adsorption rather than transport into the tissues (National Research Council, 1977a).

Cadmium

As with arsenic, the relationship between cadmium and algae has not been studied extensively because no important cadmium radionuclides are included in fallout or reactor cooling water effluent. Furthermore, the relationship between human health and cadmium exposure has only recently been firmly established (Friberg, et al., 1971).

Cadmium exists in natural waters as Cd^{2+}, $CdOH^+$, and $Cd(OH)_2$ depending on pH and Eh (Weber and Posselt 1974). In sea water, cadmium is probably in the form $CdCl^+$. One study of a pilot plant tertiary treatment/aquaculture system showed that the green algae studied concentrated cadmium to a greater extent than the diatom mixture employed (Table A-2). Furthermore, resuspension of algae contaminated with cadmium (600 ppm on a dry weight basis) into fresh sea water revealed that, after 2 hours, only 10% of cadmium had been released (Kerfoot and Jacobs, 1976). A study done on the freshwater alga *Nitella* showed that the rate and extent of cadmium uptake depended upon the amount of calcium and magnesium present. Uptake occurred faster and to a greater extent in hard water (Kinade and Erdman 1975).

Although the nature of the interaction between cadmium and algae is unknown, there is evidence that it is associated with protein. Proteins known to complex cadmium, the metallothioneins, are rich in cysteine (one out of every four amino acids) and the complex results from an interaction between cadmium and sulfhydryl groups (Kagi and Vallee 1961). These proteins are widely distributed in nature including bacteria, yeast, and marine plants.

Copper

Copper is a nutrient necessary for algal growth. In higher concentrations it is a widely used algicide (National Research Council 1977b). A fairly extensive body of literature is available on its interactions with algae. There are no radioecologic data, however, since the principal radioisotope of copper, the induced ^{64}Cu, has a half-life of only 12.8 hours. (Polikarpov 1966).

Copper is present in sea water in both particulate forms and as organic complexes (Stiff, 1971). In fresh water, copper is found as Cu^{2+}, various products of hydrolysis, $CuCO_3$ (aq), and organic complexes (Stiff, 1971; Sylva, 1976).

The specific fate of copper taken up by algae is unknown even though high concentration factors have been reported (Table A-3). Bryan (1971), working with the brown seaweed Laminaria digitata, speculated that alginates (uronic acid polymers) in cell walls and intercellular spaces act as ion exchange materials. He noted that alginates extracted from the weed exhibited affinities for divalent metals in the same decreasing order Pb> Cu> Cd> Ba> Sr> Ca> Co> Ni> Zn> Mn> Mg as the whole cells.

Laboratory studies on the effects of copper on freshwater algae have produced a variety of conclusions. Ferguson and Bubela (1974) used algal cell matter broken up by freeze drying, chopping, and/or pressure extrustion. They noted concentration factors (in terms of mass of total organic carbon) of 3.3×10^3 for Ulothrix, 4.4×10^3 for Chlamydomonas, and 9.7×10^3 for Chlorella. They speculated that metals recovered from solution by biosorption might produce sediments rich in metals. Steeman Nielsen and Wium-Andersen (1971), working with the diatom Nitzschia palea, noted that the photosynthetic rate decreased even in short-term experiments and concluded that copper probably penetrates the cells. The growth rate of this alga was not appreciably affected by copper, apparently because some of the organic matter excreted by the alga into the medium was able to bind copper and thus remove any toxic effect.

An earlier paper (Hassall 1963) compared the uptake of copper by living and by scalded cells. Cells were scalded by placing the centrifuge tubes containing them in a boiling water bath for three minutes. Copper uptake by scalded cells was found to be more rapid than uptake by living cells even though the ultimate concentration of copper is the same in both cases. Two thirds of the copper was firmly retained, even by dead cells, and neither rinses with distilled water nor potassium sulphate solution removed it.

Steeman Nielsen and Wium-Andersen (1969) reached a similar conclusion while working with Chlorella pyrenoidosa. They observed that very little of the copper bound by the alga entered the cytoplasm. They concluded that the major portion of the copper was bound in the cell walls and the slime envelopes.

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They substantiated their argument by noting that, in this organism, the cell wall and slime envelope constitute 6% of the total carbon of the cell. A later study from the same laboratory supported this view (Steeman Nielsen and Kamp-Nielsen, 1970).

That different species of algae respond differently to copper was illustrated by Button and Hostetter (1977) who showed that the diatom *Cyclotella meneghiniara* was copper sensitive while the green alga *Chlamydomonas reinhardtii* was copper resistant. The diatom removed approximately five times more copper on a cell-to-cell basis than the green alga. Cell walls were cleaned by boiling the diatom in hot nitric acid and the green alga in hot 4.5% KOH. It was found that cleaned cell walls of *Chlamydomonas* removed more copper from solution than living *Chlamydomonas* while the cleaned cell walls of *Cyclotella* adsorbed little copper compared with the living cells. The authors concluded that the cell walls of *Chlamydomonas* impart copper resistance by adsorbing copper, possibly to glycoproteins, whereas in the diatom most of the copper was associated with the plasmalemma or entered into the cell. A partial desorption of copper after 6 hours, as compared to the 3 hour values, was noted in several experiments.

Gibson (1972) noted that the blue-green alga *Anabaena flos-aquae* accumulated four to five times more copper than the green alga *Scenedesmus quadricauda* and also observed that copper (II) sulfate was more toxic to young cultures of *A. flos-aquae* than to older cultures.

Russell and Morris (1970) presented data that indicated algae can become copper tolerant. The organism under study was the marine-fouling alga *Ectocarpus siliculosis*. Foster (1977) studied *Chlorella vulgaris* and noted a non-tolerant strain accumulated five to ten times more copper than a tolerant strain, even at comparable external copper concentrations.

Stokes (1975) studied copper tolerant *Scenedesmus* isolated from a polluted lake and found specific copper-containing complexes in the nuclei. Copper was not, however, found in the cell walls or the cell membrane. A later paper (Silverberg, et al., 1976) gave data obtained from electron microscopic techniques. It was found that the intranuclear inclusions were in the form of central dense-core complexes. Copper was the only heavy metal found in the inclusion. More recent work (Stokes, et al., 1977) revealed that the inclusions were composed of copper-binding proteins with molecular weights of about 10,000 daltons. Apparently the alga detoxified copper by this method.

Lead

The relationship between lead and algae has not been extensively studied. Lead is not a fission product of nuclear explosives and is not found in reactor cooling water effluents (Polikarpov, 1966). In sea water, lead may be in the form $PbCO_3$ (aq), $PbCl^+$, $PbCl_2$ (aq), Pb^{2+}, $PbCl_3^-$ and perhaps $PbOH^+$ as well as complexed and chelated forms (Zirino and Yamamoto, 1972). In fresh water, the forms $PbOH^+$, $Pb(OH)_2$ (aq), and $Pb(OH)_3^-$ and possibly Pb^{2+} are expected to predominate (Leckie and James, 1974).

As noted before, Bryan (1971) reported that lead was bound more strongly than any other bivalent metal to alginates extracted from brown algae.

The high affinity of algae for lead is noteworthy and concentration factors as high as 7 x 10^4 have been reported (Table A-4). That different species of algae respond differently to lead was noted by Malanchuk and Gruendling (1973) who subjected five algal species to lead nitrate at 10, 20 and 30 ppm. Response was measured by light dependent fixation rates of $^{14}CO_2$. The blue-green Anabaena sp and the green desmid Cosmarium botrytis were affected by the lowest levels of lead while CO_2 fixation fell off sharply only above 10 ppm Pb for Chlamydomonas reinhardtii and the diatom Navicula pelliculosa. However, in the flagellated chrysophyte Ochromonas malhamensis, CO_2 fixation was found to increase with increasing lead concentration. The authors speculate that lead might act by physically blocking movement across membranes. They justify this statement by noting the intricate cell morphology and the high surface area-to-volume ratio in the lead sensitive Cosmarium.

Hessler (1974) studied the green marine form Platymonas subcordiformis in various concentrations of lead chloride. Noting that normal, flagellated cells survived less well than flagella-free mutants, she suggested that the flagella may be on sites of lead uptake. The mutant's resistance to lead could not be explained solely by the lack of flagella, however.

Schulze-Baldes and Lewin (1976) studied the course of lead uptake in batch assays with the marine diatom Phaeodactylum tricornutum as well as Platymonas subcordiformis. They noted a rapid increase in the lead content of cells exposed to lead and attributed the initial uptake to its adsorption to the cell surface. Cultures which had different concentrations of EDTA added immediately prior to lead treatment took up less lead than cultures without EDTA treatment, indicating that only uncomplexed lead is available for adsorption. Exposing the algae to lead for various lengths of time and then to EDTA showed that the bound lead fraction increased considerably with time of exposure, a fact explained by the translocation of lead into the cells.

Silverberg (1975) studied the ultrastructural localization of lead in the tolerant filamentous green alga Stigeoclonium tenue. The algae were exposed to various concentrations of lead nitrate for 9 days and then subjected to X-ray microanalysis and a staining procedure utilizing sodium rhodizonate. Two strategies for coping with lead exposure were noted. At low concentrations of lead, electron dense precipitates on the surface of the cell wall were noted. At exposures to higher concentrations of lead, the metal was found in the internal vacuoles of the algae. Invaginations on the cell membrane indicated that movement inwards was by pinocytosis.

Mercury

Mercury is not found in large amounts in either radioactive debris from weapons testing or reactor cooling water effluent (Polikarpov, 1966). Interest in mercury in the environment has increased dramatically since the awareness of the dangers of mercury contamination became apparent in the late 1960's and early 1970's (D'Itri and D'Itri 1977). Most of the studies involving the relationship between mercury and algae have been made since 1970.

Gavis and Ferguson (1972) reviewed the cycling of mercury through the environment. Mercury can exist in water as the native metal, in the +1 state and in the +2 state. Under various conditions of Eh and pH, mercury may exist as HgS^{2-}, $Hg(OH)_2$ (aq), Hg^{o} (aq), HgCl(OH) (aq), and HgCl (aq). The chemistry of mercury is further complicated by the affinity of the metal for sulfhydryl groups as well as its ability to be methylated. In sea water, inorganic mercury is predominantly in the form $HgCl_4^{-2}$ (Glooschenko 1969).

Glooschenko (1969) studied the marine diatom Chaetoceras costatum under conditions of light and dark with dividing, nondividing, and dead cell populations to determine the relative importance of surface adsorption versus active uptake of mercury. He found that formalin killed cells accumulated more mercury than any other population, including the dividing cell population (figured on a per cell basis). Cells in the dark were found to take up slightly more mercury than cells in the light. Dividing cells in the light showed a longer period of mercury accumulation than nondividing cells. He concluded that, while some active uptake may be present, the most important process for mercury uptake by this diatom was by surface adsorption.

Kamp-Nielsen (1971) studied Chlorella pyrenoidosa with mercury and concluded that a smaller number of sites bound mercury than copper, and mercury was bound more specifically on the cytoplasmic membrane. The bound metal ions apparently increased the permeability of the membrane since potassium and possibly phosphate leaked out of the cells. Hannan and Patoiullet (1972), while studying the effect of inorganic mercuric salts on the growth rates of four different algae, concluded that the uptake of mercury could not be accounted for by surface adsorption alone. A later paper by this group (Hannan, et al, 1973) reported exposing Phaeodactylum tricornutum to $^{203}HgCl_2$ and then extracting pigments and other cellular debris with methanol. About 2% of the bound mercury was extracted by this method, a fact attributed to intracellular transport.

Shieh and Barber (1973) studied Chlorella and noted that mercury uptake was unaffected by light. Lowering the temperature to $1^{o}C$ revealed that the influx of mercury consisted of two phases, a rapid phase completed within several minutes and a slower phase with no saturation within sixty minutes. A breakdown in cell membrane permeability was also noted. Fujita, et al. (1976) studied the effects of light, magnesium, and cyanide on the accumulation of mercury by the freshwater diatom Synedra ulna. Observing that mercury accumulation was stimulated by light as well as magnesium ions, they concluded that uptake of mercury was related to energy pathways. Cyanide at 0.01mM was found to reduce uptake to 50% of the control value, possibly by complexation with mercuric ion.

That the silica shell of the green colonial Pediastrum boryanum was responsible for mercury accumulation was suggested by Richardson, et al, (1975) who observed that skeletonized colonies took up mercury rapidly for two hours. Living cells continued to remove mercury after two hours, a fact attributed to adsorption by other cell components. Similar results were reported by Fujita and Hashizume (1975) with the freshwater diatom Synedra ulna. They noted that acid-digested silicate frustules took up mercury more rapidly than dividing cells, reaching equilibrium in a matter of minutes. Living cells took up more mercury

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but took it up more slowly, attaining a maximum level only after 24 hours. Davies (1974) reported experiments with the marine alga Isochryses galbana that indicated some mercury was translocated to cell interiors.

Burkett (1975) worked with the green filamentous alga Cladophora glomerata which he radiolabelled with methyl mercuric chloride. He found that live Cladophora adsorbed more methylmercury than the formalin-killed algal speciments. It was found that release of methylmercury from Cladophora occurred slowly, if at all. DeFilippis and Pallaghy (1976a), utilizing Chlorella, calculated that 14% of the total cell content of mercury was situated in the cell walls. Mercury-treated cultures experienced a greater increase of biomass than control cultures. This was explained by hypothesizing that mercury prevented the export of carbon compounds such as glycollates into the medium.

Since algae can recover from initial contact with mercury, several workers have searched for the mechanism of metal tolerance. Ben-Bassat, et al. (1972), working with aerated cultures of Chlorella pyrenoidosa, noted that the ability to recover from exposure to mercury salts was directly proportional to the cell density of the algal inoculum. Mercury taken up by the cells was apparently not readily available for the conversion to a more volatile form, as indicated by a minimum mercury concentration of 2 to 4 femtomoles of mercury per cell. DeFilippis and Pallaghy (1976b) developed Chlorella strains which were resistant to mercury. Resistant cells were found to volatilize much more mercury than sensitive cells when at 25C; at 2C neither strain volatilized mercury. Both strains, however, accumulated the same amounts of mercury--an amount calculated to be very close to the sulfhydryl concentration in the cells--which suggests that sulfhydryl groups have been the binding sites. No differences in sulfhydryl concentration between resistant and sensitive strains were noted. Ben-Bassat and Mayer (1977) determined that the reducing factor or factors were extracellular agents and that the volatile mercury species released was Hg, as indicated by its entrapment on PdCl paper, a specific test for mercury. The reducing factor or factors were found to have a molecular weight of about 1200, to be heat stable at 85C for short periods, and to give a positive reaction for reducing sugars.

Nickel

There has been very little research on the relationship between nickel and algae, due in part to its low concentrations in natural waters (National Research Council, 1975). Furthermore, the principal radioisotope of nickel (^{63}Ni) though possessing a long half-life, emits a beta-particle of such low energy that it is of no great ecological concern (Polikarpov, 1966).

Equilibrium forms for nickel have been calculated for sea water and typical fresh water by Sibley and Morgan (1975). In sea water, nickel was found to be principally in the ionic state, while in fresh water the carbonate species were the most important, followed by the free ion and then the hydroxide. Some of the nickel (40%) was computed to be adsorbed on particulate matter.

Alginates extracted from the brown seaweed *Laminaria digitata* were found to have a lower affinity for nickel than for any other divalent ion except magnesium (Bryan 1971). Concentration factors for nickel can be found in Table A-6.

Stokes (1975) studied algae isolated from lakes polluted with both copper and nickel and compared them with related algae from laboratory stocks. She was able to obtain concentration factors of up to about 1×10^4 for the isolated strain of *Scenedesmus* studied. Concentration of nickel was found to occur over a narrow pH range. A number of other factors, including calcium concentration, were found to influence the uptake of nickel. No information as to ultrastructural localization was given, however.

Zinc

Zinc, like copper, has been widely studied for its effects on algae. Early work emphasized the algal concentration of ^{65}Zn because of its long half life (245 days) and energetic gamma ray. Concentration factors established for zinc are among the highest for the metals reported here and all species studied have been able to concentrate zinc to a considerable extent (Table A-7). Like copper, zinc is a required micronutrient for algae, while in higher concentrations it becomes toxic (Polikarpov, 1966).

Equilibrium calculations reveal that in surface sea water zinc is most likely present in the form of a chlorocomplex (Zirino and Yamamoto, 1972; Sibley and Morgan, 1975). In fresh water the principal form of zinc was calculated to be the hydrated ion, but carbonate, sulfate, and hydroxyl species are present to some extent (Sibley and Morgan, 1975).

Early studies on the uptake of zinc by the marine diatom *Nitzschia closterium* were conducted by Chipman, et al. (1958). Their work indicated that this species accumulated zinc far in excess of metabolic needs. A culture with an initial concentration of 43×10^7 cells per liter removed all but a trace of the zinc (added as the sulfate) within 24 hours, with 80% removal being attained within the first hour. A concentration factor of about 5×10^4 was calculated using isotope concentration in equal cell and medium volumes as the basis for comparison. EDTA, when added to the culture, prevented the uptake of zinc. Distilled water rinses removed little of the zinc, although repeated rinses with increasingly concentrated zinc solutions removed up to 43% of the cell bound zinc isotope.

Bachman and Odum (1960) used ^{65}Zn to trace the uptake of zinc by brown algae under conditions of light and dark. Noting that no uptake was observed in the samples incubated in the dark and that light-incubated samples showed a correlation between uptake and oxygen production, the authors concluded that a metabolic process was involved in the uptake process. In fact, ^{65}Zn uptake was suggested as a possible assay for primary production.

A series of three papers by Gutknecht (1961, 1963, 1965) provided an alternative explanation for the correlation between zinc uptake and oxygen production. The first paper in this series reported that freshly killed thalli (undifferentiated plant bodies) of the green macroalga *Ulva lactuca* sorbed zinc more

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rapidly than live thalli. Furthermore, a pronounced pH dependence of both light and dark zinc sorption, with six times as much zinc adsorbed from media buffered at pH 9 as at pH 7, led to the conclusion that the apparent correlation between uptake and photosynthesis was due to the changes in pH caused by metabolic activity. The second paper in this series extended the investigation to the red alga *Porphyra umbilicalis* and the brown alga *Laminaria agardi*, with essentially the same results. Studies with metabolic inhibitors (phenylurethane and uranyl nitrate) showed that they had a relatively slight effect on uptake rates. The last paper utilized several more species of algae. Results from this study are included as a part of Table A-7.

Bryan (1971), when discussing the affinity series of divalent cations for alginates and heavy metals, noted that the position of zinc in the series (higher only than magnesium) seemed anomalous since zinc was highly concentrated by the brown algae from which the alginates were extracted. Bryan therefore postulated that other materials such as cell proteins probably governed the distribution of zinc.

A series of papers by Austrian workers (Schuster and Broda, 1970; Matzu and Broda, 1970; Findenegg et al, 1971) reported on investigations utilizing *Chlorella pyrenoidosa*. In the first paper, experiments utilizing cell walls of the algae were described. Characterization of the chemical nature of the cell wall by a pH titration and the determination of adsorption isotherms revealed that two kinds of sites were available for zinc binding. The authors, using information available on the biochemical composition of the cell wall, concluded that phosphate groups, perhaps those associated with lipids, were primarily responsible for zinc adsorption. The second paper described the short-term uptake of zinc under culture conditions of light and air (representing an energy rich situation) or dark and nitrogen (an energy poor situation). Under ideal conditions (pH 5, 5×10^{-6} M Zn, 5×10^{-3} M Ca), about 20% of the zinc was removed from solution after 20 hours but under minimal energy conditions only 5% was removed. The authors concluded that the short-term uptake was a physicochemical mechanism, with the zinc remaining in the cell walls. The last paper in this series described a chemostat for determining uptake, something that the authors felt was advantageous since extracellular metabolites could not accumulate in the medium. Here it was found that at 30C the uptake of a 1.5×10^{-6} zinc solution (buffered at pH 6.25) showed a strong light dependency, with uptake about doubling within an hour after the light was turned on. At 5C, the light-dependent uptake of zinc was practically nil.

Kempner and Miller (1972), while they were determining the minimal medium for *Euglena gracilis*, observed that the initial concentration of zinc(10^{-7} M) was depleted completely within a few hours. Even though no further zinc was available, cell growth continued at the same rate until the population had increased a hundred fold. Falchuk, et al. (1975) reported that in zinc-deprived *Euglena gracilis*, cell size increased, rate of cell division decreased, and amount of intracellular paramylon increased.

Parry and Howard (1973) studied the marine alga *Dunaliella tertiolecta* and observed the same pH dependence on uptake that Gutnecht noted. The pH of the culture medium varied over 1.4 pH units as a result of photosynthesis or respiration, a factor used to explain the pH dependence of zinc uptake. The authors noted that this alga does not possess a cell wall; proteins (specifically the amino groups) were suggested as binding sites.

DeFilippis and Pallaghhy (1976a, b) utilized *Chlorella* in studying uptake of zinc and the mechanisms of resistance to the metal. In the first report, it was noted that zinc was taken up at the same initial rate at 2C and 25C, even though the higher temperatures resulted in about one-third more zinc being taken up after 2 hours. The zinc (1 mM as the chloride) was postulated to be bound to the free spaces in the cell wall. A long-term slower uptake of zinc was also noted. The second paper in the series showed that resistance to zinc was induced by *Chlorella* after a long-term (50 days) exposure to a 1 mM $ZnCl_2$ solution. The resistance seemed to be due to a zinc exclusion phenomenon since sensitive cells removed far more zinc than resistant cells.

Styron, et al, (1976) used a factorial design to determine the effects of 8 temperatures (6 to $40^{\circ}C$) and 10 salinities (3.5 to 44.0 g/l) on the uptake and concentration of zinc for 6 marine algae. Concentration factors for zinc ranged from 1 to 2.2×10^4 for one organism (*Achnanthes*, Table A-7) and showed clearly the importance of environmental factors in determining uptake rates.

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SECTION 5

MATERIALS AND METHODS

ALGAE

Sources and Types of Algae

The selection of representative algae to screen for heavy metal uptake was dictated by several factors. First, it was desired to have species of algae from the major taxonomic groups (cyanophytes, chlorophytes, etc.). Second, it was necessary to select algae exhibiting various morphological features (colonial, filamentous, unicellular, etc.). Third, the species selected had to be amenable to laboratory culture in one of a relatively few culture media. Fourth, the algae had to grow to a sufficient biomass in a relatively short period of time. Fifth, the algae selected had to grow in a strict inorganic medium because such a medium would result in a relatively low risk of bacterial contamination of the cultures.

Table 2 lists the algae selected for possible use, the sources from which the algae were obtained, information about their taxonomic and morphological features, and a qualitative assessment of their growth in the three media employed in the study. It should be noted that not every media-algal combination was attempted. If satisfactory growth was achieved in Bristol's medium, no other medium was employed.

All algal cultures were unialgal, a fact checked occasionally by microscopic examination. Any culture found contaminated by other algae was discarded and, when possible, a new culture was started from agar slants. The only exceptions were cultures derived from samples taken from Cazenovia and Onondaga Lakes. One-ml subsamples of each lake were cultured in both Bristol's and the diatom medium. No attempt was made to quantify or identify the algae in these mixed cultures.

Culture Media

It has been said that there are perhaps as many media and modifications used for algal growth as there are active phycologists today (Nichols 1973). Certainly the beginning worker in phycology is apt to be confused by the wide variety of recipes available for culture media. Therefore, it was decided to use a limited number of media and accept the fact that not all algae could be cultured successfully.

TABLE 2. SOURCES AND CULTIVATION OF ALGAE

Algae	Source *	Type	Growth on media† B	D	SW
A. Baccillariophytes (diatoms)					
Navicula pelliculosa	UTEX 668	Pennate diatom	-	+	-
B. Chlorophytes (green algae)					
Chlamydomonas sp.	Dr. C. Kuehnert Syracuse University	Unicellular,2 flagella	+		
Chlorella pyrenoidosa	"	Unicellular	+		
Chlorotyllium sp.	Dr. N. Lazaroff SUNY Binghamton	Filamentous	+		
Kirchnerella sp.	"	Colonial, enclosed in gelatinous matrix	+		
Mougeotia sp.	UTEX 758	Filamentous, planktonic	+		
Scenedesmus obliquus	UTEX 2016	Colonial, 4-8 cells, planktonic	+		
Spirogyra sp.	Dr. N. Lazaroff	Filamentous, planktonic	+		
Ulothrix fimbrinata	Dr. N. Lazaroff	Filamentous, benthic	+		
Zygnema	UTEX 923	Filamentous, enclosed by mucliaginous sheath	+		

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TABLE 2. (continued)

Algae	Source*	Type	Growth on media† B	D	SW
C. Cyanophytes (blue-green algae)					
Gleotrichia sp.	Dr. Lazaroff	Colonial	+		
Nostoc muscorum A	"	"	+		
Nostoc F	"	"	+		
Nostoc muscorum H	"	"	+		
Nostoc L	"	"	+		
Nostoc muscorum W	"	"	+		
Nostoc 31	"	"	+		
Oscillatoria sp.	"	"	+		
Nostoc 586	"	"	+		
Schizothrix calcicola	"	"	+		
D. Mixed Cultures					
Cazenovia Lake			+	+	
Onondaga Lake			+	+	

*University of Texas Culture Collection and Identification.
†B=Bold's medium; D=diatom medium; SW=soil water medium.
+ = success
- = failure

Three media were selected for use: Bold's modification of Bristol's medium (Nichols 1973), a freshwater diatom medium (Darley and Volcani 1971) and a soil-water medium. Table 3 displays information about the first two media.

The soil-water medium was prepared by placing approximately 1/2 in. of commercial potting soil and 300 ml of distilled water in a 500-ml Ehrlenmeyer flask, adding a pinch of $CaCO_3$, plugging the flask with cotton and cheesecloth, and then steaming for approximately an hour on two successive days.

All chemical reagents used were analytical grade. The water was distilled water. Final pHs were 6.6 for the Bristol's medium, 8.3 for the diatom medium, and variable alkaline pHs (depending on the amount of $CaCO_3$ added) for the soil-water medium.

Culturing Techniques

Agar Slants--

Cultures obtained were either axenic or bacterized unialgal. Those that were axenic were maintained on agar slants composed of Bristol's medium solidified with 1.5% agar. Transfers were accomplished about every 4 months using a loop and streaking onto fresh slants. Those algae which could be maintained in this manner were: *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Gleotrichia*, all of the *Nostoc* strains, and *Schizothrix calcicola*. These slants were maintained at 20°C.

Liquid Cultures--

Seventy-five ml of culture medium were dispensed into 125ml Erlenmeyer flasks that were then plugged with cotton and cheesecloth and sterilized by autoclaving. After cooling, 1 ml of an existing liquid culture was pipetted into the fresh medium. Transfers were made every 10 to 20 days. Cultures were sometimes inoculated from agar slants by transferring a loop of cells from the solid to the liquid medium.

The flasks were maintained on a gyrorotatory table (New Brunswick Scientific Co.) at 120 oscillations per minute. Light, at 560 lux, was supplied by cool-white fluorescent tubes on a 16 hour light, 8 hour dark basis. Temperature was maintained at 20°C.

Certain strains selected for further study were grown on a larger scale. Thirteen one-liter glass bottles were filled with 10 liters of nutrient medium and sterilized by autoclaving. Laboratory compressed air was first filtered through cheesecloth and then passed through glass tubing into the nutrient medium at a rate sufficient to cause the suspension of the algae. Illumination (at 430 lux) was supplied by a bank of Life Line fluorescent tubes on the same light-dark regime.

Determination of Dry Weights

Algal dry weights were obtained by using two techniques. The first method employed involved suction filtration of a known volume (usually 5 or 10 ml) through a 4.25 cm diameter Whatman #2 filter paper disc. The filter paper and a corresponding disposable aluminum weighing dish had previously been dried at 103 ^{o}C and weighed to the nearest o.1 mg on an analytical balance. The filter disc, with the filtered algae, was then placed in its corresponding weighing dish and redried, again at 103 ^{o}C, to constant weight. The difference between final and initial weights divided by the volume filtered yielded dry weight on a mg/ml basis.

There were several problems with this technique. First, the suction apparently lysed some samples as the filtrate was sometimes green. This was particularly true for samples of unicellular algae such as Chlorella and Chlamydomonas. Second, those algae with mucilaginous sheaths blocked the filter, leading to filtration time of over an hour for a 5 ml sample. This was especially true for the blue-green algae.

Because of these problems, precision was poor. For example, filtering 10 ml of a 10 day culture of Schizothrix calcicola yielded a dry weight of 0.15 mg/ml, while 20 ml of the same culture yielded a value of 0.28 mg/ml. Twenty ml of a 10 day culture of Oscillatoria yielded a value of 0.22 mg/ml, while 10 ml of the same culture showed a value of 0.15 mg/ml.

The above lack of precision led to a modification that was both more rapid and more precise. Aluminum weighing pans were marked and dried at 103 degrees C, cooled in a desiccator and weighed. A sample of the algal culture, usually 5 or 10 ml, was then pipetted directly into the pan which was then returned to 103 degrees C and dried to constant weight. An example of the improved precision is demonstrated by the following data. Four replicate samples (5 ml) of a Scenedesmus culture were determined, with the dry weights (after correction) found to be 1.38 mg/ml, 1.26 mg/ml, 1.32 mg/ml and 1.38 mg/ml. Equal volumes of sterile media were handled in the same manner to provide a correction for the salt content of the media. All dry weights after January 1978 were determined by the improved technique.

Cell Counting Techniques

Cell counts, when done, were made using the Utermohl technique, as described by Schwoerbel (1970). One ml of the culture was diluted with 19 ml of distilled water. Lugol's solution (10 g KI, 5 g I_2 and 5 g sodium acetate in 70 ml of water) was added dropwise until the color resembled that of a fine cognac. The resulting mixture was then allowed to stand for 1 day, after which the vessel was shaken and 10 ml poured into a 10 cc tubular chamber, mounted on a plate chamber covered with a plexiglass cap and allowed to sediment for about 4 hours. The tube was then slid off the plate chamber, replaced by a cover glass and the plate chamber, with the sedimented algae, placed on an inverted microscope (Unitron BU-13) and counted using a Whipple disc of 1.3×10^{5} nm^{2}. Five fields were counted and the average used to calculate the number of organisms per ml.

METAL UPTAKE STUDIES

Whole Culture Studies

In an attempt to learn something of the time scale of metal uptake for use in designing the microtiter assay technique described later, several preliminary studies were performed using various algal-heavy metal combinations. Table 4 lists the algal culture used, their age in days, and the cation(s) with which they were tested.

TABLE 3. CULTURE MEDIA USED

A. Bold's Bristol Solution

Stock Solution		Used for one liter of medium	Final Concentration	
			mg/1	mM
Macronutrients				
1. $K_2HPO_4 \cdot 3H_2O$	3 g/400 ml	10 ml	75	0.43
2. KH_2PO_4	7 g/400 ml	10 ml	175	1.29
3. $NaNO_3$	10 g/400 ml	10 ml	250	2.94
4. NaCl	1 g/400 ml	10 ml	25	0.43
5. $MgSO_4 \cdot 7H_2O$	3 g/400 ml	10 ml	75	0.30
6. $CaCl_2 \cdot 2H_2O$	1 g/400 ml	10 ml	25	0.17
Micronutrients			ug/1	μM
7. EDTA KOH	50 g/1 31 g/1	1 ml	50 31	171.09 553.00
8. $FeSO_4 \cdot 7H_2O$ H_2SO_4	4.8 g/1 1.0 ml/1	1 ml	4.98	17.9
9. H_3BO_3	11.42 g/1	1 ml	11.42	184.7
10. $ZnSO_4 \cdot 7H_2O$	8.82 g/1	1 ml	8.82	30.7
$MnCl_2 \cdot 4H_2O$	1.44 g/1		1.44	7.3
$Na_2MoO_4 \cdot 2H_2O$	1.00 g/1		1.00	4.9
$CuSO_4 \cdot 5H_2O$	1.57 g/1		1.57	62.9
$Co(NO_3)_2 \cdot 6H_2O$	0.49 g/1		0.49	16.8

(continued)

TABLE 3. (continued)

B. diatom medium

Stock solution		Used for one liter of medium	Final concentration	
Macronutrients			mg/1	mM
1. $Ca(NO_3)_2 \cdot 4H_2O$	4 g/400 ml	10 ml	100	0.42
2. $K_2HPO_4 \cdot 3H_2O$	3 g/400 ml	1.8 ml	13.5	0.059
3. $MgSO_4 \cdot 7H_2O$	3 g/400 ml	3.5 ml	25	0.10
4. Na_2SiO_3 $9H_2O$	4 g/400 ml	10 ml	100	0.47
5. Na_2CO_3	0.8 g/400 ml	10 ml	20	0.18
Micronutrient mixture			μg/1	μM
6. H_3BO_3	0.568 g/1	1 ml	0.568	9.16
$ZnCl_2$	0.624 g/1		0.624	4.58
$CuCl_2 \cdot H_2O$	0.268 g/1		0.268	1.58
$Na_2MoO_4 \cdot 2H_2O$	0.252 g/1		0.252	1.04
$CoCl_2 \cdot 6H_2O$	0.42 g/1		0.42	1.76
$MnCl_2 \cdot 4H_2O$	0.36 g/1		0.36	1.82
$FeSo_4 \cdot 7H_2O$	2.50 g/1		2.50	8.99
Sodium tartrate $2H_2O$	1.76 g/1		1.76	7.65

TABLE 4. ALGAL-METAL COMBINATIONS USED IN BATCH STUDIES

Alga	Age (days)	Cations							
		As(III)	As(V)	Cd	Cu(II)	Hg(II)	Ni(II)	Pb	Zn
Chlamydomonas	12	+	+	+	+	+	+	+	+
Chlorella	12			+	+		+	+	
Spirogyra	12		+	+	+	+	+	+	
Ulothrix	12	+	+	+	+		+	+	+
Ulothrix	20	+			+		+	+	
Gleotrichia	12				+		+	+	
Gleotrichia	20	+							
Gleotrichia	40	+							
Nostoc 31	40	+	+						
Nostoc 586	12	+	+	+	+	+	+	+	
Nostoc 586	20	+							
Oscillatoria	12	+	+	+	+	+	+	+	+
Oscillatoria	20	+							

The basic procedure was to take approximately 75 ml of algal culture and transfer it to centrifuge tubes. After centrifugation, the supernatant was discarded and the cell mass resuspended in 50 ml of any one of the following solutions: lead nitrate, cadmium nitrate, copper sulfate, nickel nitrate, sodium arsenite, sodium arsenate, zinc chloride, or mercuric nitrate. All solutions except the mercuric nitrate were at a concentration of 20 μg/ml with respect to the metal. Mercury was present at 50 μg/ml. Sodium nitrate was added to all heavy metal solutions to attain a final cation concentration of 0.04M.

The cultures were left unagitated in the dark. No attempt was made to monitor or control the pH in the flasks except for a final pH reading. Three milliliter samples were withdrawn at 3, 12, 18 and 24 hours, after which the cultures were filtered by gentle suction and washed with 5 ml of distilled water and 5 ml of 0.1 N HNO_3. Dry weights of the algal mass were determined. The four samples and the two washes were analyzed for heavy metal concentration on a Perkin-Elmer model 603 atomic absorption spectrophotometer using the appropriate hollow cathode lamps. All analyses were performed according to the manufacturers recommendations (Perkin-Elmer 1976).Results were compared to control solutions (no algae) subjected to the same sampling and analytical regime.

Microtiter Assay Techniques

Introduction--

The large array of variables affecting metal uptake by algae made a systematic study of removal mechanisms difficult. Therefore, an important first step in this study was the adaptation of microtitration equipment and techniques, for use with radionuclide tracer methods. What follows is the description of the original technique as it was employed (Protocol I) and then the improved methodology (Protocol II) developed during the study. First, however, information common to both techniques will be given.

Radionuclides--

Table 5 lists information about the radionuclides used in this phase of the study. Isotopes were diluted to the necessary volume with distilled water, vigorously mixed to ensure a uniform nuclide concentration and dispensed to the plates as described below. After sampling, the nuclide to be counted was transferred to a 15 x 115 mm glass counting tube and counted on a Nuclear Chicago gamma scintillation counter (Model 4216) equipped with a NaI crystal detector.

Preparation of Algae for Microtiter Assay--

Algal cultures were centrifuged for five minutes at 2000 x g. The supernatant then was discarded and the algal cells were resuspended in distilled water to one-fourth the original volume. The four-fold concentrate was then dispensed into the designated wells by a micropipette. Filamentous algae as well as some colonial forms could not be dispensed through the micropipetters. These cultures were placed in an Oster blender (John Oster Manufacturing Co., Milwaukee, Wisconsin) and blended at high speed for 90 seconds. Robinson (1968) has shown that 90 seconds produced filaments of reasonably homogeneous lengths with a minimum of cell damage. Cultures subjected to this regime included *Chlorotyllium*, *Spirogyra*, *Ulothrix*, *Mougeotia*, *Zygnema*, *Gleotrichia* and *Nostoc* 31.

Microtiter Assay Technique (Protocol I)--

Microtitration equipment--Microtitration equipment used included microtitration plates, micropipettes, and a multi-microdiluter. The first series of experiments utilized 96 wells (8 rows by 12 columns), disposable, V-bottom, acrylic plates (Flow Laboratories, Rockville, Maryland, #76-364-05). Each well had a capacity of about 0.2 ml. Micropipettes used were either reusable polypropylene pipettes with stainless steel tips, or disposable pipettes made from plastic tubing with plastic tips. Each delivered drops of 0.025 ml at a claimed precision of ±2%. The reusable pipettes were used to dispense inert solutions; the disposable pipettes were used for radionuclide solutions. The multi-microdiluter (Cooke Engineering Co., Alexandria, Virginia) allowed the simultaneous use of six 0.025 ml microdiluters that had been trimmed to 90 mm in length. The microdiluters allowed the transfer of 0.025 ml samples from the wells to the counting tubes.

Procedure-- The initial microtitration assay technique (Protocol I) is illustrated in Figure 1. Each well in a plate was filled with 4 drops from a micropipette. Every well received a drop of Bristol's solution (diluted 1/10 with distilled water), a drop of buffer, and a drop of radionuclide solution. Experimental wells received a drop of algal cells and control wells received a drop of distilled water. Dilution of the algal cells in the wells was therefore 1:4, restoring the algae to the same concentration as the culture from which they were derived. The plates were then incubated in a dark, humidified environment at room temperature (21°C) for three hours. After this labelling period the plates were centrifuged for 20 minutes at 2000 x g in a floor model centrifuge using carriers designed for the purpose. The six-place multi-microdiluter was used to transfer 0.025 ml from the supernatant of individual wells to 5 ml of 20 mM EDTA in scintillation tubes. The microdiluters were prewetted and blotted to improve their uniformity of sample uptake; occasionally they were flamed with alcohol (Conrath, 1972). After the supernatant was transferred, the diluters were rinsed in a flowing stream of water. After the plate was sampled, the tubes were counted.

TABLE 5. RADIONUCLIDE DATA

Nuclide	Source*	Anion	Solvent	Half-life †	Mass ††	Activity
^{203}Hg	NEN	NO_3^-	5 ml of 0.5N HNO_3	46.6 d	0.84 mg	6 MC/mg 5 MC total
^{109}Cd	NEN	Cl^-	5 ml of 0.5N HCl	450 d	0.59 mg	3.37 MC/mg 2 MC total
^{210}Pb	A	NO_3^-	5 ml of 3N HNO_3	21 y	0.03 mg	2 MC total
^{65}Zn	UMRR	Zn^-	7 ml of 2N HCl	243.6 d	47 mg	3 MC total
^{76}As	UMRR	As_2O_3	5 ml of 0.5N HNO_3	26.5 h	28.2 mg	20 MC

* NEN = New England Nuclear Corp.; A = Amersham Corp.; UMRR = University of Missouri Research Reactor.

† Handbook of Chemistry and Physics.

†† Information supplied by isotope manufacturer.

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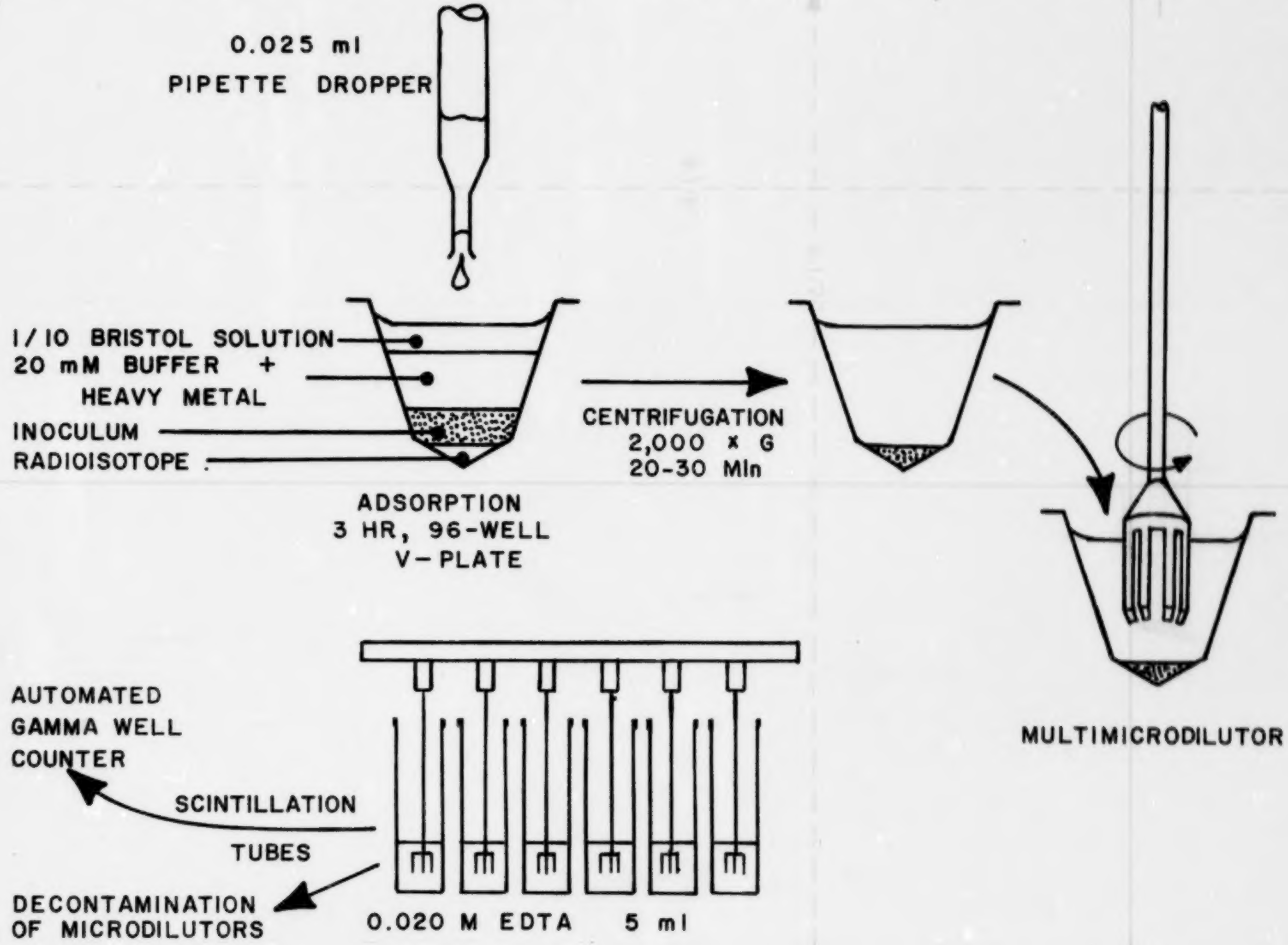
0.025 ml
PIPETTE DROPPER
1/10 BRISTOL SOLUTION
20 mM BUFFER +
HEAVY METAL
INOCULUM
RADIOISOTOPE
ADSORPTION
3 HR, 96-WELL
V-PLATE
CENTRIFUGATION
2,000 x G
20-30 Min
MULTIMICRODILUTOR
AUTOMATED
GAMMA WELL
COUNTER
SCINTILLATION
TUBES
DECONTAMINATION
OF MICRODILUTORS
0.020 M EDTA 5 ml

Figure 1. Details of microtiter technique (Protocol I).

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Statistical methods--The information derived from the gamma counter (i e., the counts per minute remaining in the supernatant) was used as the basis for the data reduction and statistical analysis employed during this phase of the study. The mean and standard deviation of the control and experimental wells were determined. For a given experiment there were usually 12 control wells and several groups of 6 experimental wells. The means were used to compute the percentage removal by use of the formula

$$\%\ \text{Removal} = \frac{\bar{X}\ \text{control} - \bar{X}\ \text{experimental}}{\bar{X}\ \text{control}} \times 100$$

where $\bar{X}$ control is the control mean and $\bar{X}$ experimental the test mean. Significance of the differences between experimental and control means was determined by use of Student's t test (Steel and Torrie 1960).

Microtiter Assay Technique (Protocol II)--

Equipment--Though the micropipettes used were the same as those used in the original technique, different plates and supernatant collection device were used for improved methodology. The plates were 96-well (8 rows by 12 columns), disposable U-bottom, polystyrene plates (Flow Laboratories, Rockville, Maryland, #76-311-05). Besides having U-bottom wells, these plates were considerably stiffer than those used previously. Each well had a capacity of approximately 0.25 ml.

The Titertek™ supernatant collection apparatus used included harvesting frames, a press, an alignment rack, transfer tube frames, and a transfer fork (Flow Laboratories, Rockville, Maryland). The harvesting frames were disposable plastic holders containing 48 cellulose acetate absorption cartridges, each with a glass-fiber filter disc. The adsorption cartridges were spaced in 4 rows and 12 columns corresponding to wells in the plates.

The press was used to push the 48 absorbing cartridges simultaneously into the microtitration plates to a uniform depth and the alignment rack aligned the transfer tube frames to the same spacing as the harvesting frame. The transfer tubes (strips of twelve plastic tubes) were designed to receive the absorbing cartridges. The transfer fork was used to push the cartridges from the frame into the transfer tubes.

Procedure--The technique was developed originally for clinical laboratories which use immunoassays based on the release of ^{51}Cr from erythrocytes (Herschberg et al., 1977). In this work it was used to assay the unadsorbed radionuclide remaining in the incubation mixture. The technique is detailed in Figure 2. After the labelling period, the plastic holder containing the 48 cellulose acetate adsorption cartridges was placed over 48 wells of a plate. The cartridges were pressed down into the wells and held for about 2 seconds, a time sufficient to absorb 0.080-0.085 ml of supernatant. The glass-fiber filter at the end of the cartridge had a diameter slightly larger than the microplate well and therefore formed a seal between itself and the well wall. After the cartridge had been lifted off the plate, the filter discs were left behind, preventing the adsorption of any algal cells. The cellulose acetate cartridges (still in the

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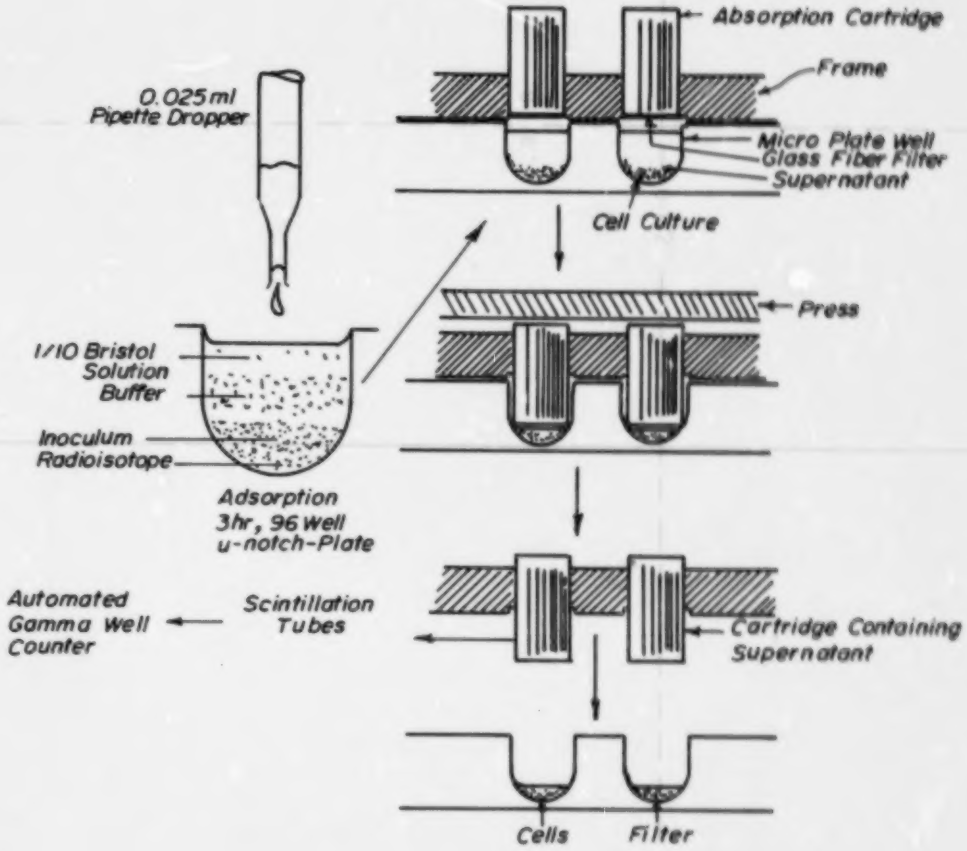
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Frame
Micro Plate Well
Glass Fiber Filter
Supernatant
Cell Culture
0.025 ml
Pipette Dropper
1/10 Bristol
Solution
Buffer
Inoculum
Radioisotope
Adsorption
3hr, 96 Well
u-notch-Plate
Press
Automated
Gamma Well
Counter
Scintillation
Tubes
Cartridge Containing
Supernatant
Cells
Filter

Figure 2. Details of improved microtiter technique (Protocol II).

harvesting frame) were then placed over a rack of 48 transfer tubes and were pushed down, one cartridge to a tube, with the plastic transfer fork designed for the purpose. The plastic transfer tubes were broken off and dropped into the glass scintillation tubes for gamma counting. The process was then repeated for the remaining 48 wells on the plate. The plastic tubes inside the scintillation vials were nearly vertical and showed only minute differences in counting geometry. Some initial problems with reproducibility of counts were corrected by drying the cartridges (either in their transfer rack or scintillation tubes) for about 3 hours at 60 degrees C.

Statistical methods--The increased capacity of the improved system (Protocol II) called for an improved method of data collection, reduction and analysis. These ends were achieved by means of a computer program developed specifically for this project.

Data developed from Protocol I showed the desirability of conducting the metal uptake studies over a wider pH range than first employed. Therefore, a control plate (8 rows of 12 wells each) was used with rows maintained at pH 3, pH 4 and pH 10 by various buffers. There were no algae in the control plate wells; as shown in Figure 3, this control plate was used in conjunction with several experimental plates. Each experimental plate was divided into two regions each with 8 rows and 6 columns. The rows were maintained at pH 3 to pH 10 by buffers. A droplet of algae was placed in each of the 6 wells in the pH 4 to 10 rows, while distilled water rather than algae was used in the 6 wells at a buffered pH 3. The pH 3 wells thus served as an interplate control (IPC).

Data obtained from the gamma counter were used in conjunction with the algal dry mass and concentration of the isotope to calculate the following:

i) Removal efficiency:

$$\frac{\bar{X}\ \text{control} - \bar{X}\ \text{experimental}}{\bar{X}\ \text{control}}$$

ii) Accumulation co-efficient

$$\frac{\mu\text{g metal removed}}{\text{g algal dry weight}}$$

iii) $$\frac{(\mu\text{g metal removed})/(\text{g algal dry weight})}{(\mu\text{g metal})/(\text{ml total medium volume})}$$

Significant differences between residual activity in the experimental and control wells were determined by use of Dunnett's t criterion, which is designed to test several treatment means against a common control mean. This technique utilizes an estimate of the pooled variance and provides one t value by which significance is determined for each control-experiment mean pair. The calculated value of t was determined by use of the formula:

$$t = \frac{d'}{sd}$$

where d' is the difference between 1 experimental mean and the control mean and sd is the joint standard error of the difference, determined by use of the formula

$$sd = \sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

where n_1 and n_2 are the number of replicates in the experimental and control groups (6 and 12 respectively, as shown in Figure 3) and s^2 is the error mean square for the entire experiment (Steel and Torrie, 1960).

Data were collected and stored in an IBM 370 computer. Upon completion of data entry, the statistical program was initiated and the results printed. It is important to note that comparisons between control and experimental means were all done at the same pH; there were then, in effect, eight experiments being analyzed simultaneously, one at each pH.

Experimental controls were maintained in two ways. First, the mean counts from each experimental plate were compared to the control mean at the same pH. The pH 3 rows contained no algae and were utilized as interplate controls (IPC, see Figure 3). The IPC data were used to test the hypothesis that all the plates had been handled similarly. If the IPC data from an experimental plate were not significantly different from the control mean at pH 3, it was assumed that the experimental plate had been handled in the same manner as the control plate and the data at each pH were then accepted for analysis. If the IPC data for an experimental plate were different from the control mean at pH 3, all the data on that plate were rejected.

The second control procedure was to use one-half of an experimental plate with distilled water rather than algae at all pHs. The means for each row from the half plate were compared to each row from the entire control plate. In this manner, unusual trends across the entire pH range could be detected.

Summary--

The improved technique had several advantages over the original technique. The newer technique was faster because the plates did not have to be centrifuged, and sampling a plate took 30 seconds compared to about 10 minutes for the original method. In addition, there was no danger of the cells being taken up (as occasionally happened when the microdiluters disturbed the algal pellet in the original method).

Most importantly, as the results sections will show, precision of counts between sample wells was increased dramatically. This was probably due, in part at least, to the elimination of any cross contamination between wells. The improved method was superior also in radiological safety, because all the liquid to be counted was held in the absorbing elements, minimizing chances for contamination. The use of disposable absorbing elements also reduced the volume of radioactive wastes that had to be disposed of as a result of decontamination rinsing and washing.

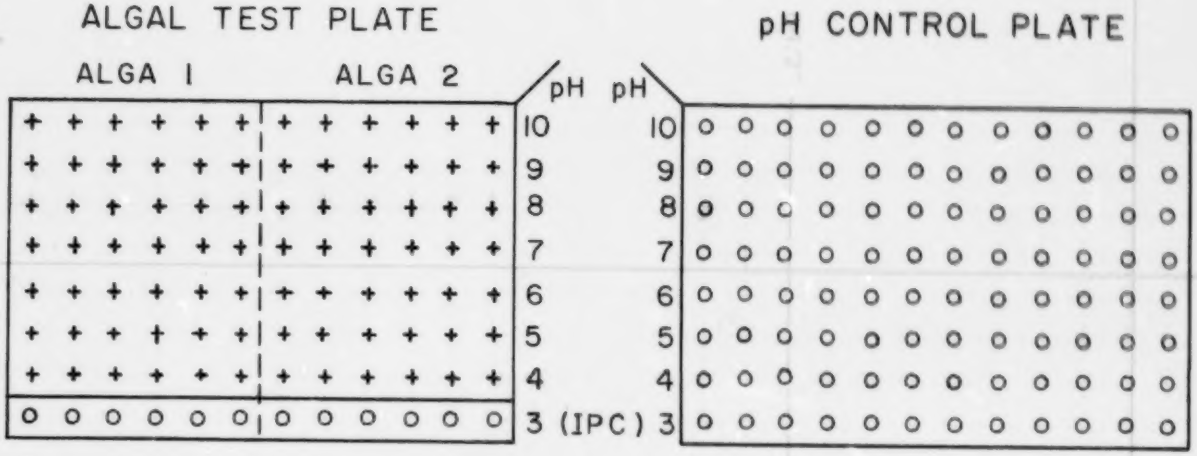
ALGAL TEST PLATE
ALGA I
ALGA 2
pH
10
9
8
7
6
5
4
3 (IPC)
pH CONTROL PLATE
pH
10
9
8
7
6
5
4
3

Figure 3. Experimental procedure as used in improved microtiter assay technique (Protocol II) for comparing the removal of heavy metal radionuclides by 2 algae at different pH. + wells with algal inoculum; o wells without algae; IPC = interplate control.

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SECTION 6

RESULTS

ALGAL GROWTH

Algal growth was sufficient for 20 species to provide a representative selection of algae for the experiments. Some species proved impossible to culture and others exhibited unusual rates of growth. Table 2 reveals that no growth was obtained from any form in soil-water media. At first this seems surprising because some workers considered this a general medium, suitable for many forms (Nichols 1973; Starr 1964). The choice of African violet potting soil was probably unfortunate because this soil is high in humus. Starr (1964) states that success with this medium depends on the selection of a garden soil with a medium humus content.

A few algal forms were characterized by unusual growth rates. Mougeotia and Zygnema grew only slowly and to a low final biomass. Nostoc 31, Nostoc W, and Gleotrichia exhibited a long lag phase. For each of these forms, inoculation was followed by about 15 days of apparent inactivity. After this period, growth to a useful final biomass was rapid. For these forms, then, no young culture data are available.

BATCH STUDIES

General Discussion

The results of the batch studies are presented in Figures 4 through 11 and Table 6.

The figures show the concentration of metals in the sample as a function of the time the samples were withdrawn from the culture. It should be noted that, for reasons of clarity, not all data points are shown on the figure.

The table includes the dry weight determined for each algal culture used, the age of the culture (in days), the concentration of metal found in the four timed samples, and the concentrations in both the distilled water and acid rinse. It will be recalled that all metals had an initial concentration of 20 μg/ml except mercury, which was present at 50 μg/ml.

Arsenic III--

The data show no obvious trends in the removal of arsenic III. The highest removal (about 35%) was accomplished by Chlamydomonas, which also had the lowest biomass (0.12 mg/ml).

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TABLE 6. METAL UPTAKE - BATCH STUDIES

Metal	Culture	(age)	Biomass (mg/ml)	Samples (μg metal/ml) 3 hr	12 hr	18 hr	24 hr	DW*	AR†
Arsenic III	Chlamydomonas	(12)	0.12	17	19	15	13	1	2
	Ulothrix	(12)	0.50	18	18	18	20	1	2
	Ulothrix	(20)	0.18	17	18	17	15	3	1
	Gleotrichia	(20)	0.58	18	18	18	19	6	3
	Gleotrichia	(40)	0.92	19	19	20	18	6	3
	Nostoc 31	(40)	0.58	15	15	16	18	4	0
	Nostoc 586	(12)	0.34	17	17	15	16	1	1
	Nostoc 586	(20)	0.56	16	17	18	16	1	1
	Oscillatoria	(12)	0.22	18	18	19	15	4	0
	Oscillatoria	(20)	0.44	21	18	17	14	3	0
	Control			20	20	20	20		
Arsenic V	Chlamydomonas	(12)	0.10	20	21	21	18	2	3
	Ulothrix	(12)	0.24	18	18	18	20	2	2
	Nostoc 31	(40)	0.44	18	17	18	18	4	0
	Nostoc 586	(12)	0.40	18	19	17	16	4	2
	Oscillatoria	(12)	0.20	20	22	19	18	3	0
	Control			22	22	21	20		
Cadmium	Chlamydomonas	(12)	0.06	12	11.2	13.2	13.6	3.4	1.7
	Chlorella	(12)	0.04	12.8	11.2	12.0	12.8	3.2	1.6
	Spirogyra	(12)	0.12	13.2	13.2	12.8	13.6	2.9	0.5
	Ulothrix	(12)	0.34	11.6	10.0	10.4	10.0	2.5	4.0
	Gleotrichia	(12)	0.06	12.4	12.8	13.2	10.8		
	Nostoc 586	(12)	0.22	9.6	12.4	12.4	10.8	3.6	4.8
	Oscillatoria	(12)	0.14	13.2	11.6	12.8	12.4	2.9	1.7
	Control					15.2	16.0	3.6	2.2

(continued)

TABLE 6. (continued)

Metal	Culture	(age)	Biomass (mg/ml)	3 hr	12 hr	18 hr	24 hr	DW	AR
				Samples(µg metal/ml)					
Copper	Chlamydomonas	(12)	0.08	13.6	16.8	19.2	14.4	5.4	0.3
	Chlorella	(12)	0.08	16.0	16.0	16.0	15.2	1.2	1.9
	Spirogyra	(12)	0.26	16.8	16.4	15.2	14.4	3.6	2.0
	Ulothrix	(12)	0.20	12.0	19.2	15.2	14.8	2.9	2.3
	Gleotrichia	(12)	0.14	13.2	10.8	14.0	11.6		
	Nostoc 586	(12)	0.20	7.2	9.2	8.4	16.0	5.2	3.6
	Oscillatoria	(12)	0.26	17.2	18.4	17.6	16.8	3.7	1.6
	Control				18.8	17.2	16.8	3.2	2.0
Lead	Chlamydomonas	(12)	0.08	2.2	1.6	3.3	4.2	1.7	16.0
	Chlorella	(12)	0.08	11.9	1.3	2.9	9.4	4.1	13.7
	Spirogyra	(12)	0.20	14.4	15.4	14.7	14.1	2.8	4.0
	Ulothrix	(12)	0.22	1.1	4.3	3.4	1.6	0.7	30.1
	Nostoc 586	(12)	0.28	6.0	3.7	4.8	4.0	0.8	28.3
	Oscillatoria	(12)	0.32	4.8	3.1	2.2	1.1	0.5	21.7
	Control				14.0	17.2	12.0	0.8	4.0
Mercury	Clamydomonas	(12)	0.16	14	8	8	9	4	23
	Spriogyra	(12)	0.36	29	14	11	12	4	20
	Nostoc 586	(12)	0.36	6	4	6	9	2	50
	Oscillatoria	(12)	0.24	26	20	19	19	2	31
	Control			34	32	34	36		

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TABLE 6. (continued)

Metal	Culture	(age)	Biomass (mg/ml)	Samples(μg metal/ml) 3 hr	12 hr	18 hr	24 hr	DW	AR
Nickel	*Chlamydomonas*	(12)	0.04	19.6	18.8	19.2	18.4	4.5	2.5
	Chlorella	(12)	0.08	19.6	16.8	19.2	20.0	2.8	1.3
	Spirogyra	(12)	0.32	16.4	16.8	18.0	18.4	4.5	2.5
	Ulothrix	(12)	0.08	16.0	18.8	19.2	18.4	4.5	2.5
	Nostoc 586	(12)	0.10	17.6	18.4	20.8	19.6	4.9	1.4
	Oscillatoria	(12)	0.20	18.0	19.2	18.8	15.2	4.5	2.5
	Control			22.4	23.2	21.6	24.0	5.0	1.1
Zinc	*Chlamydomonas*	(12)	0.12	17.2	14.0	14.0	16.8	2.8	2.8
	Spirogyra	(12)	0.20	14.0	13.6	10.8	15.6	2.8	8.0
	Ulothrix	(12)	0.20	16.0	25.2	16.0	20.4	2.8	2.4
	Nostoc 586	(12)	0.38	15.6	12.8	11.6	15.6	2.8	10.4
	Oscillatoria	(12)	0.28	17.6	9.2	16.8	15.6	3.2	0.8
	Control			19.6	22.8	18.4	16.8		

* Concentration in distilled water rinse.

† Concentration in HNO_3 rinse.

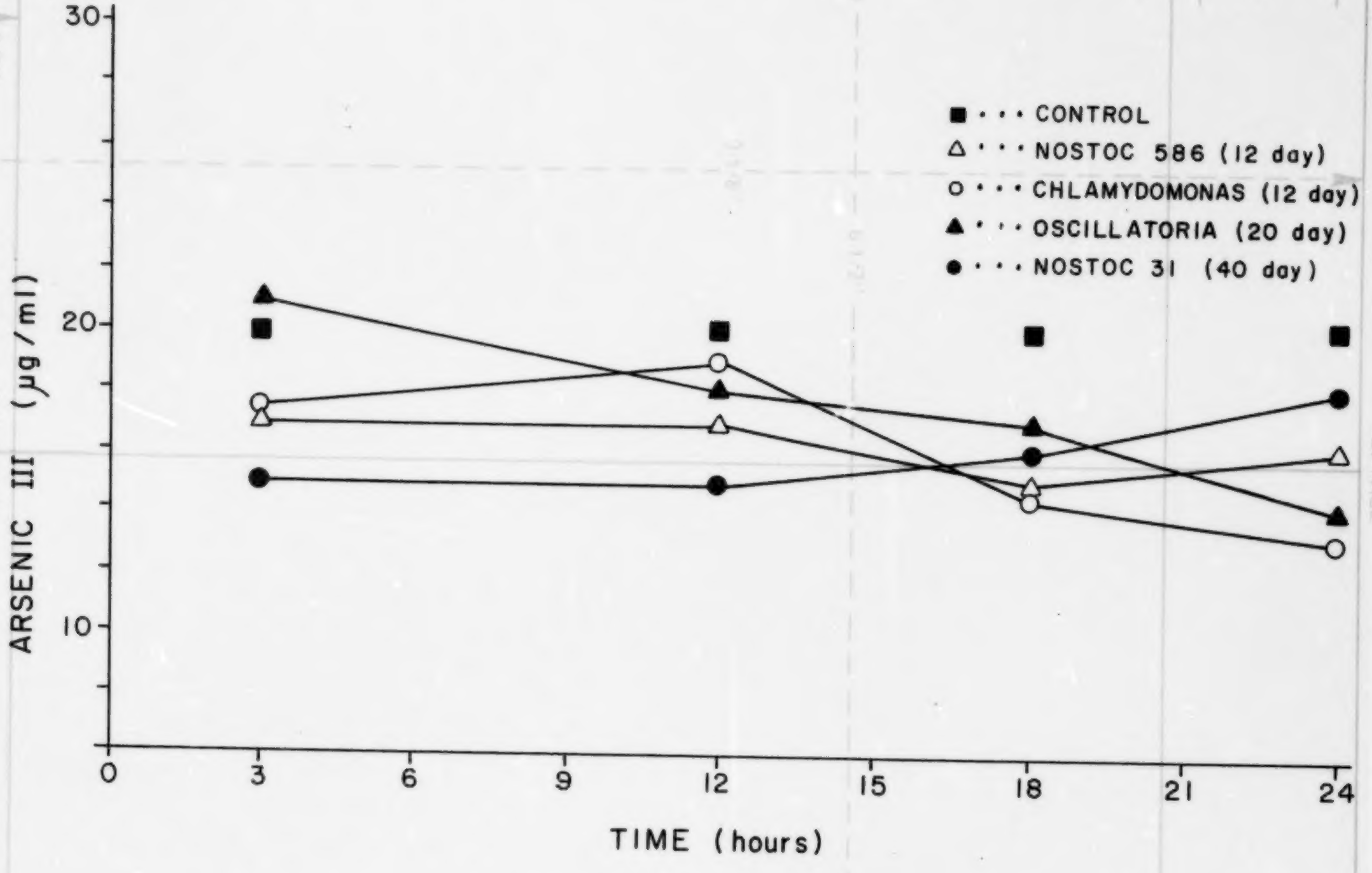

Figure 4. Uptake of arsenic (III) by various algae.

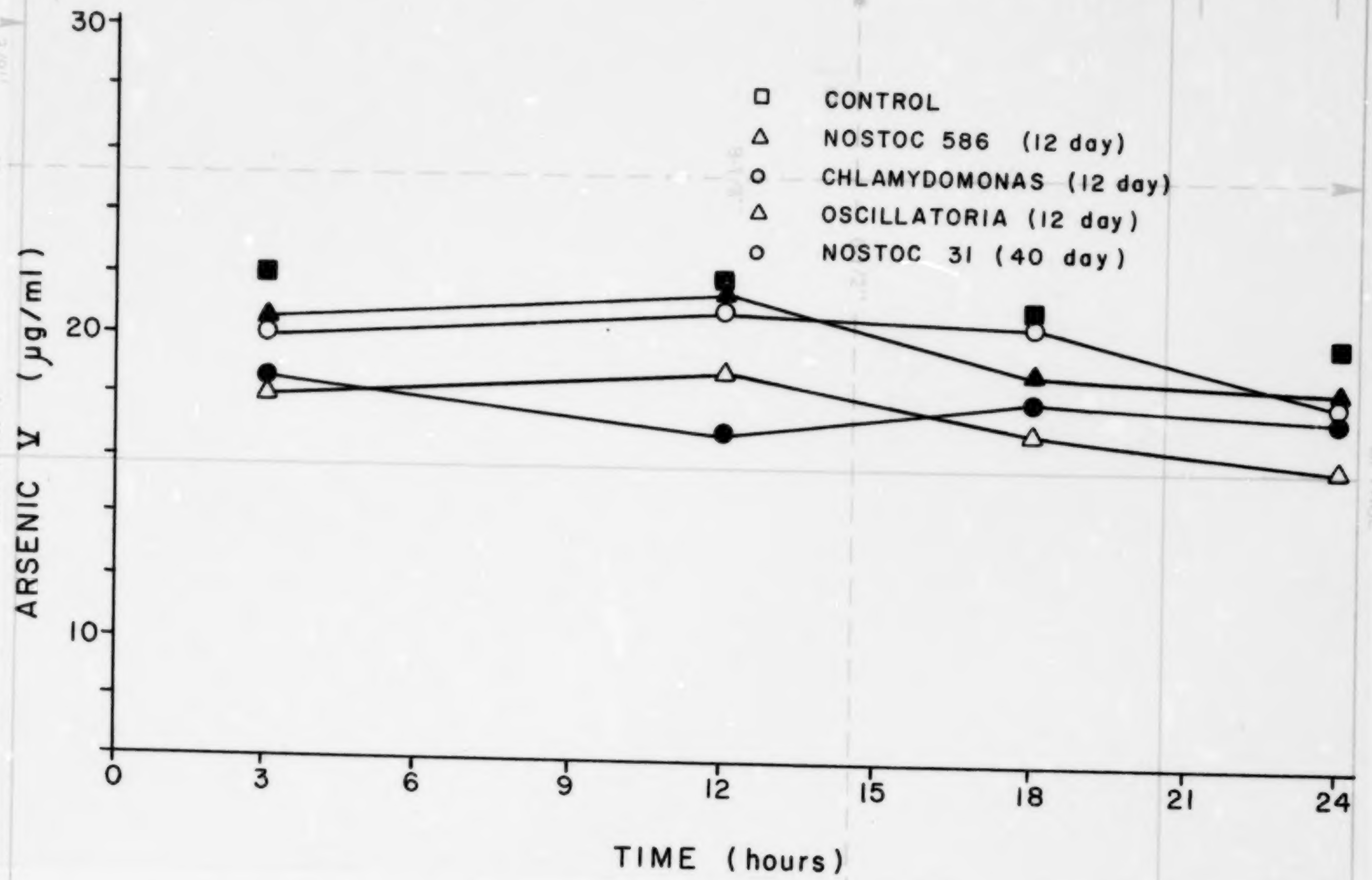

Figure 5. Uptake of arsenic (V) by various algae.

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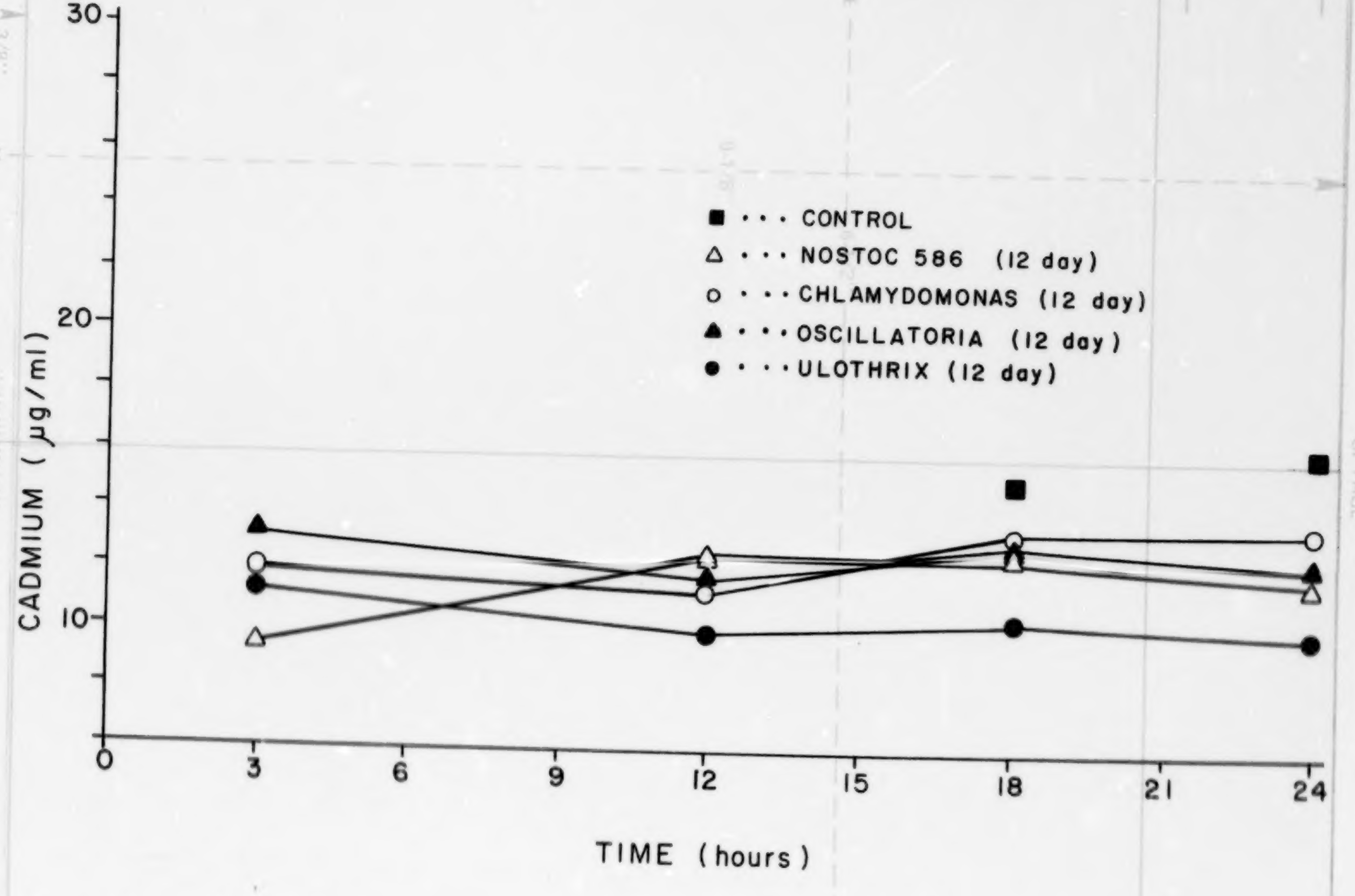

Figure 6. Uptake of cadmium by various algae.

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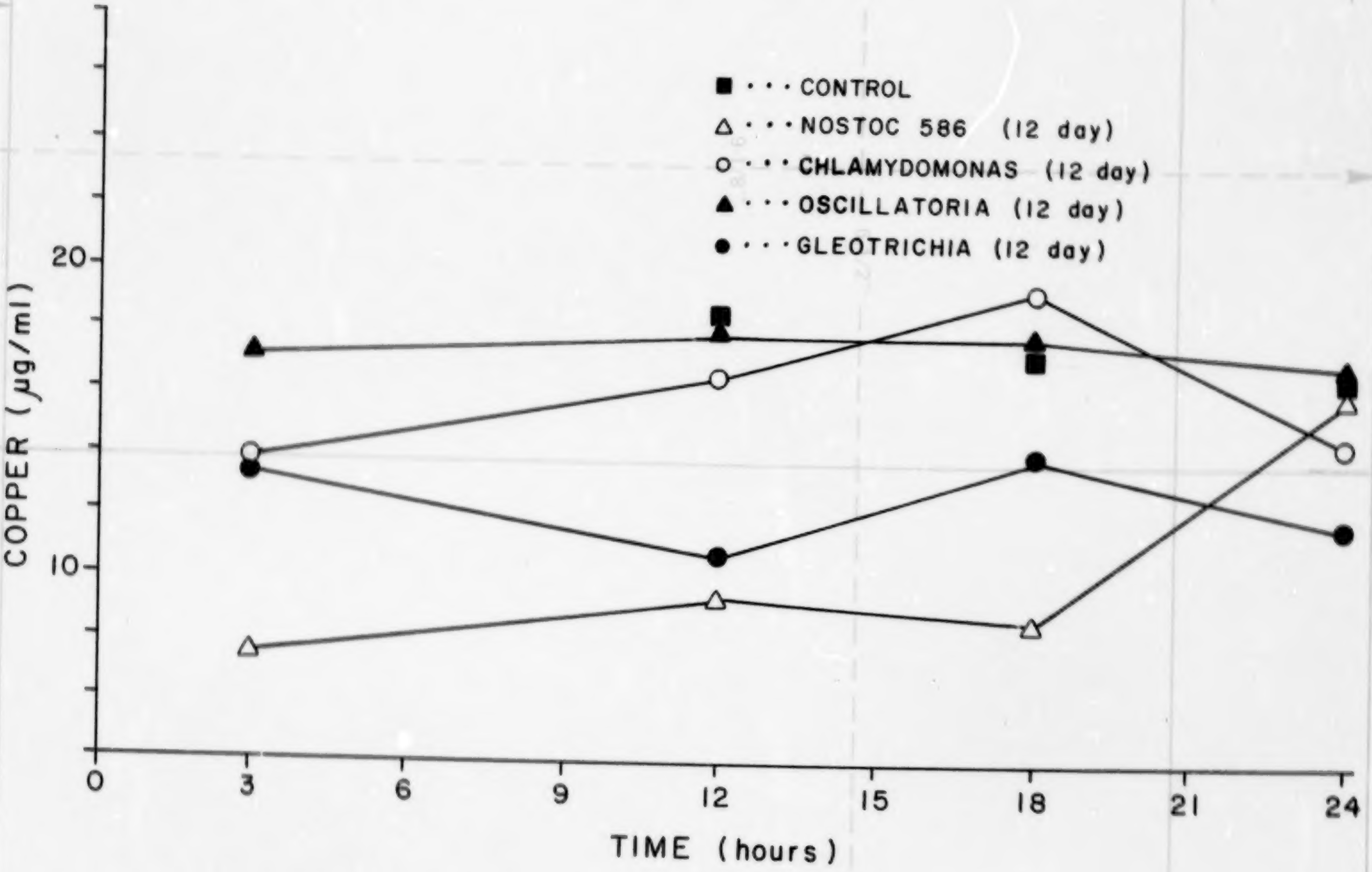

Figure 7. Uptake of copper by various algae.

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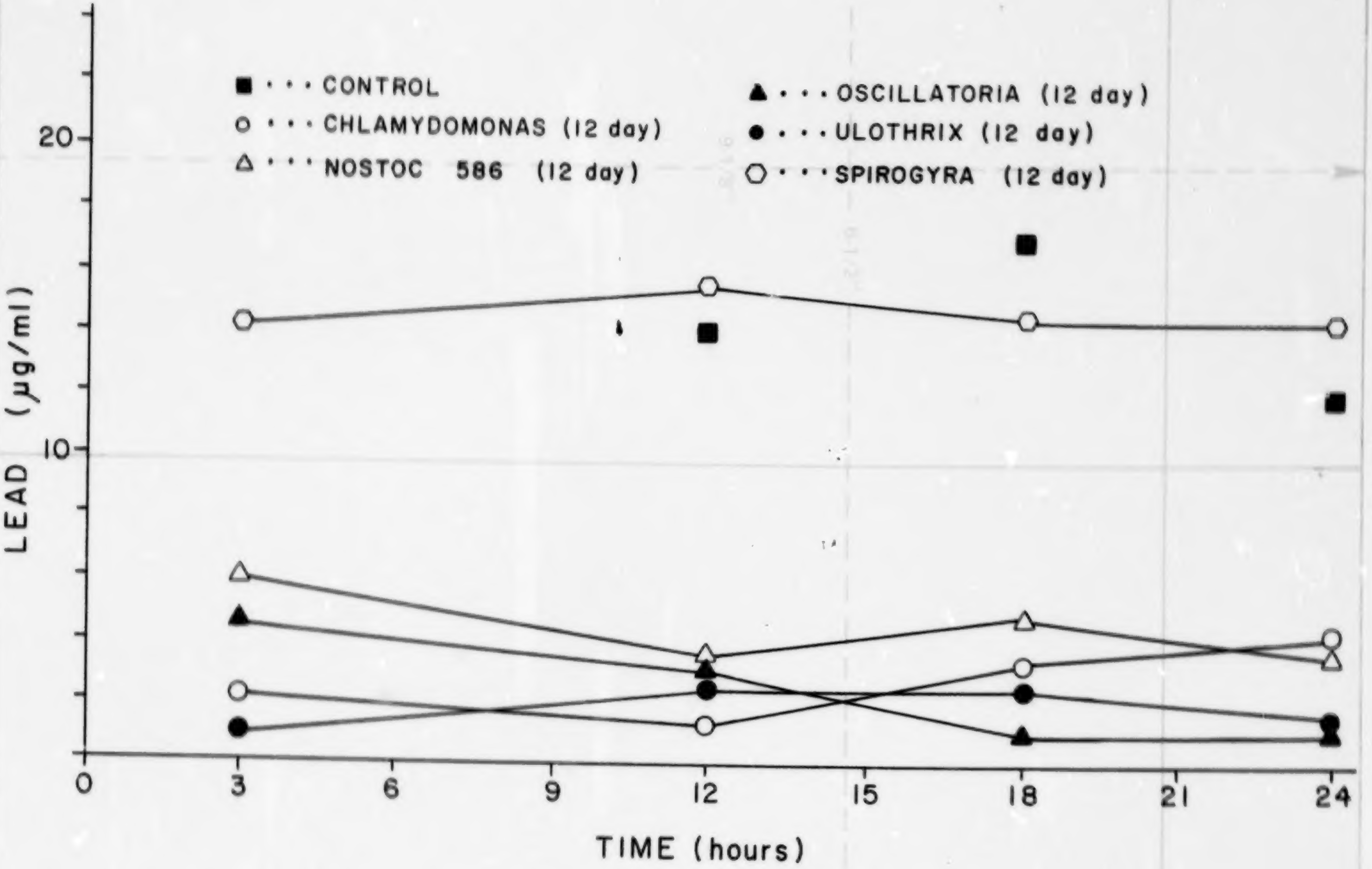

Figure 8. Uptake of lead by various algae.

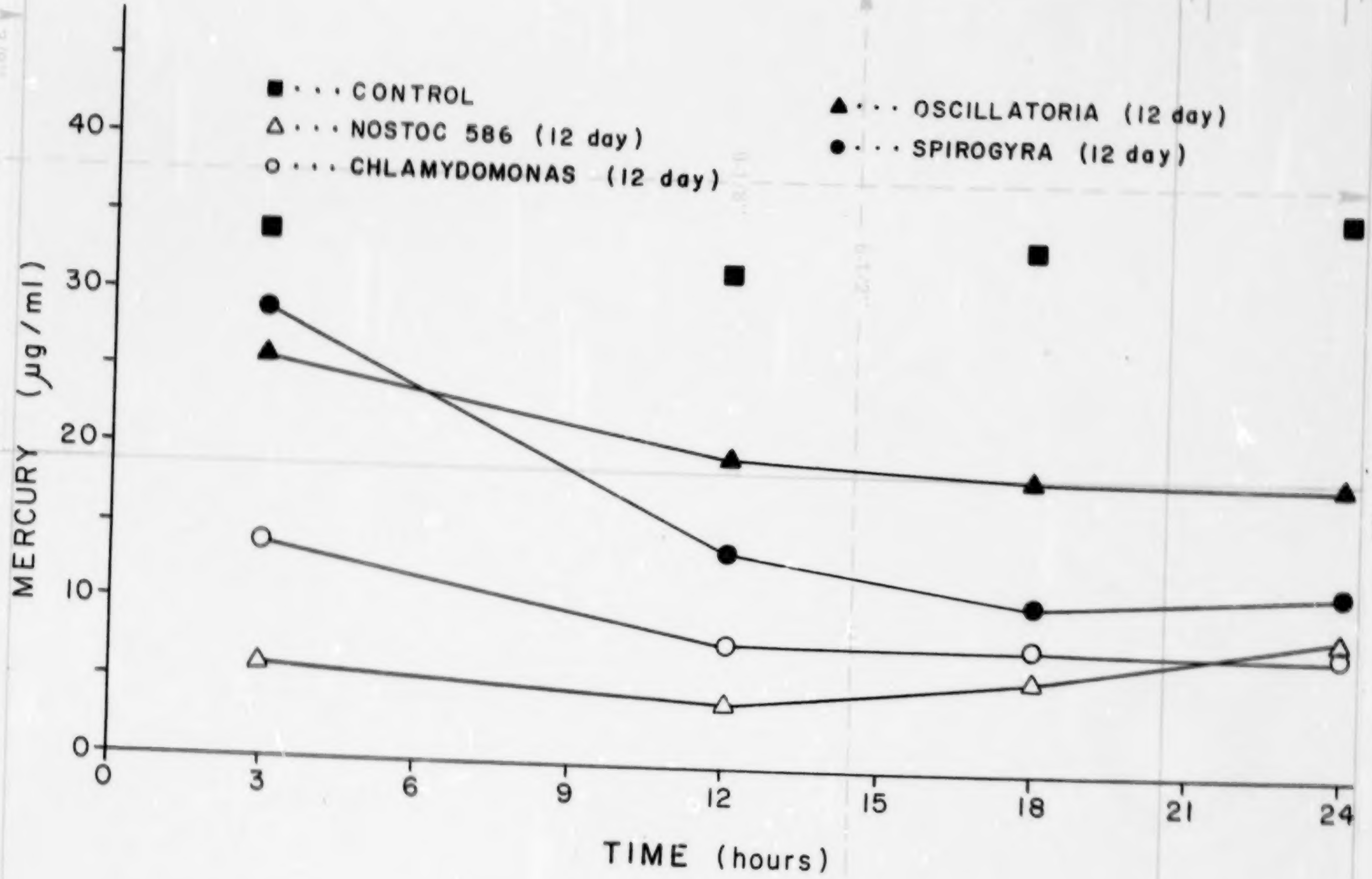

Figure 9. Uptake of mercury by various algae.

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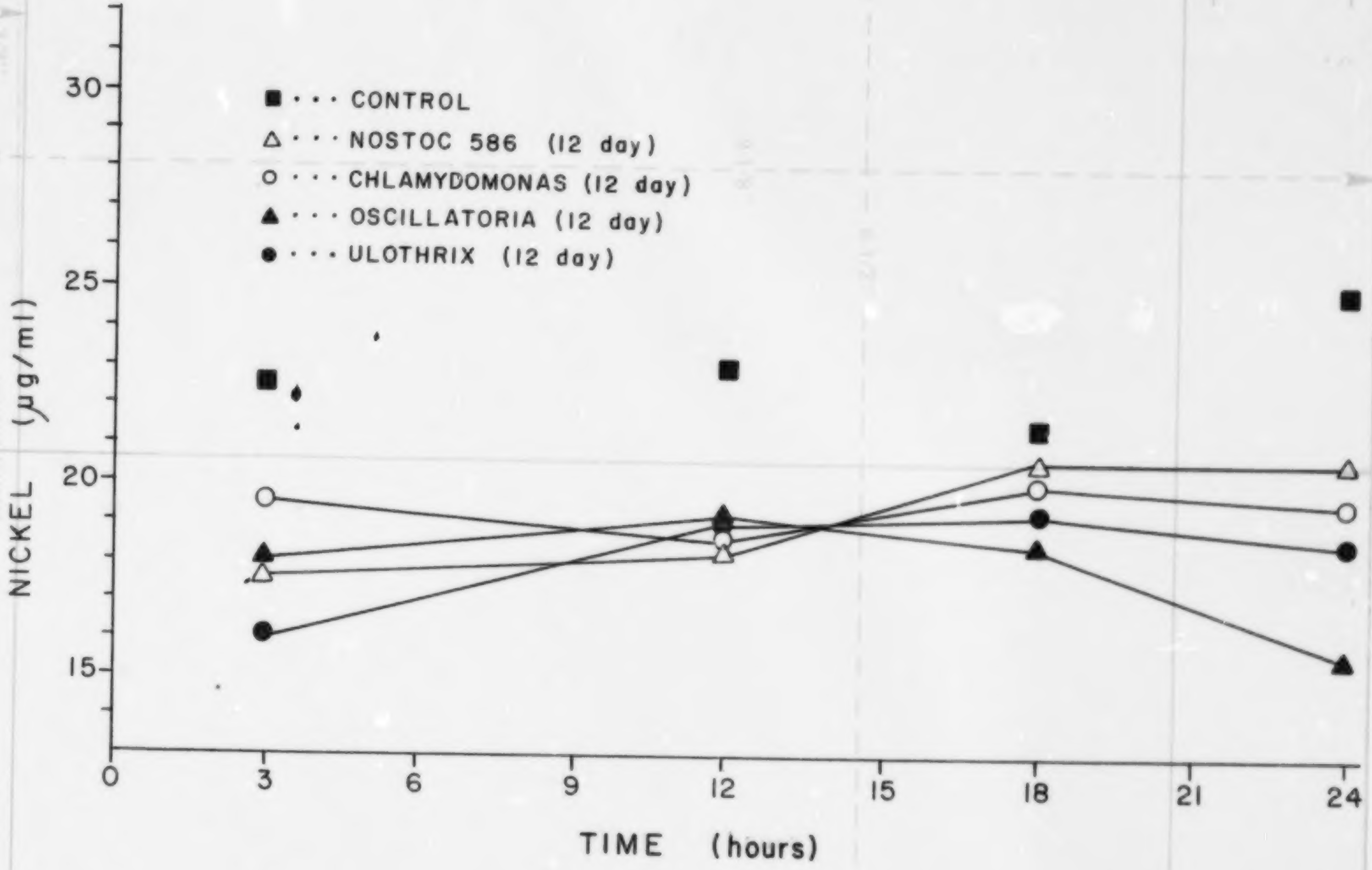

Figure 10. Uptake of nickel by various algae.

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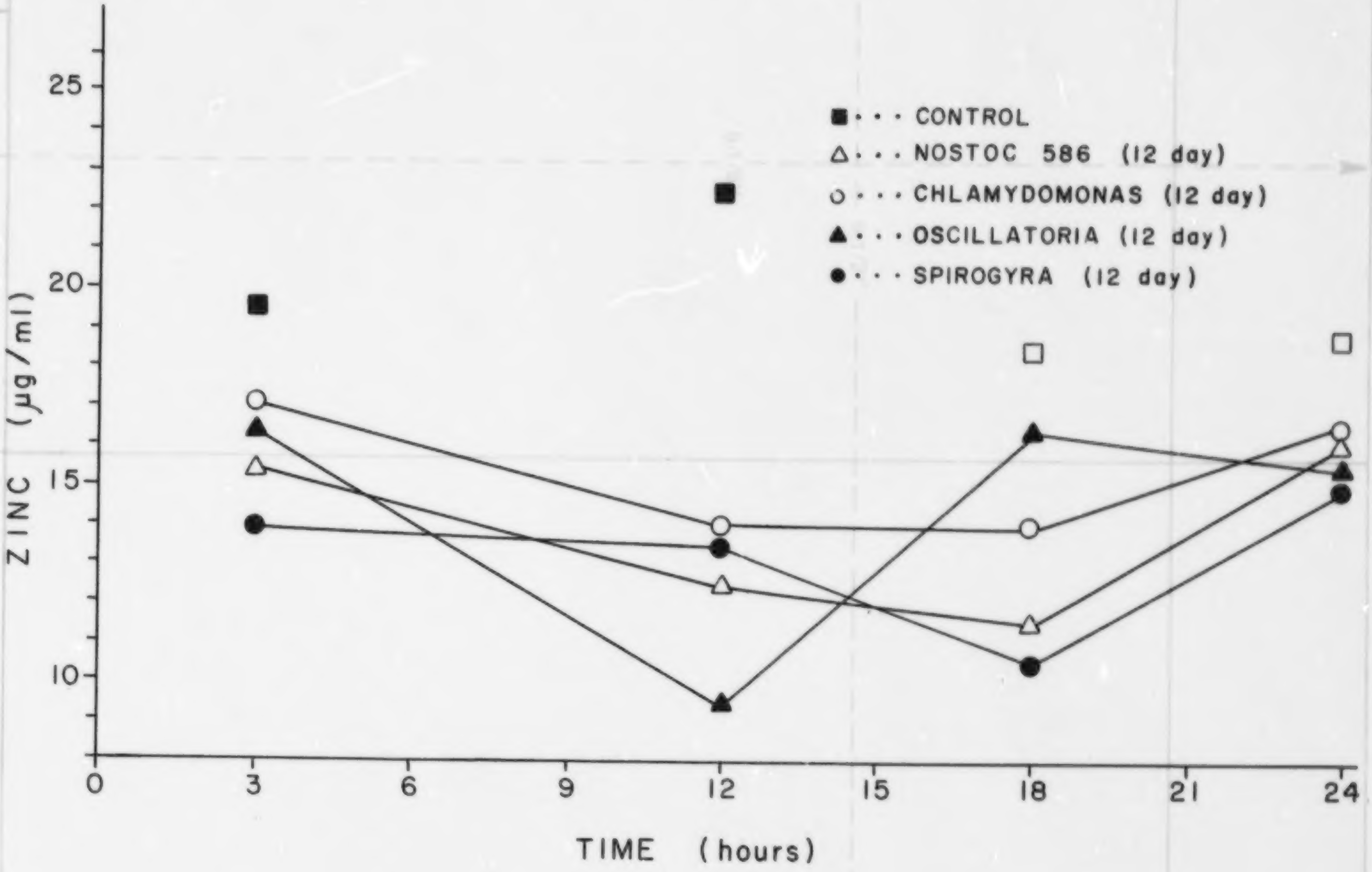

Figure 11. Uptake of zinc by various algae.

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Arsenic V--

There are no obvious trends in the removal of arsenic V. Most experimental values are too close to the control value to be considered significantly different. The lowest experimental value (16 µg/ml, compared to a control value of 20 mg/ml) was attained by Nostoc 586, which had among the highest biomass (0.40 mg/ml).

Cadmium--

Cadmium was likewise not dramatically removed by the algae used in this part of the study. The lowest experimental values (about 10 µg/ml) were achieved by Ulothrix, which had the highest biomass (0.34 mg/ml). That the acid rinse for both Ulothrix and Nostoc 586 (with the next lowest experimental value) had high concentrations of cadmium indicates a surface adsorption mechanism for cadmium removal.

Copper--

The highest removal of copper (about 30% compared to the controls) was attained by Gleotrichia, which had a biomass of about 0.14 mg/ml. This alga, though usually a dark brown mass of filaments, turned greenish on exposure to copper.

Lead--

Lead appeared to be removed by all the forms studied in this experiment, with removal (compared to the 18 hour control) of from 15% for Spirogyra to about 87% for Oscillatoria. The green filamentous Ulothrix showed high values of lead removal, and the morphologically similar Spirogyra showed values little different from the controls, even though both algae had nearly identical biomasses. The high values of lead in the acid rinses suggests again a surface adsorption.

Mercury--

The most dramatic removal of mercury was accomplished by Nostoc 586, which had an experimental value of 6 µg/ml as compared to the control value of 34 µg/ml after only three hours. Again the values of mercury in the acid rinses were high.

Nickel--

Though all algae seemed to exhibit some removal of nickel (17 to 37% after 24 hours), no trends could be discovered from the data.

Zinc--

The algae that seemed to accumulate zinc were Spirogyra (with 41% removal after 18 hours) and Nostoc 586 (with 37% removal during the same length of time). Again, the acid rinses of these two algae had high values of zinc, suggesting surface adsorption sites.

RESULTS OF MICROTITER ASSAYS

Initial Techniques - Protocol I

General Discussion--

Several variables were studied by means of the original technique utilized in this study. These variables will be discussed in turn. First, however, information common to all experiments will be presented.

The radionuclides used in this part of the study were ^{76}As, ^{109}Cd, ^{203}Hg, ^{210}Pb, and, ^{65}Zn. Dilutions of the stock solution were made to provide radioactive counts appreciably above the background. The pH was maintained at 6.5 and 8.0 by the use of Tricine buffer (N-tris (Hydroxymethyl) methylglycine) at 0.020 M for pH 6.5 and 8.0; pH 5 was attained by addition of HCl to maintenance medium.

Determination of Error--

An important first step in the development of any technique is the determination of the error associated with the technique. To this end, a plate was made up with ^{210}Pb, water, and buffer to 3 different pHs, and the plate was sampled by two operators. The results are shown in Table 7.

TABLE 7. RELIABILITY OF MICRODILUTERS FOR SAMPLING RADIONUCLIDE (LEAD 210) IN MICROCULTURE PLATES

Operator	pH	n	$\overline{X}$ (cpm)	σ_x	CV*
A	5.0	14	1442	162	0.11
	6.5	13	1480	333	0.23
	8.0	14	1393	135	0.10
B	5.0	12	1401	195	0.14
	6.5	12	1402	236	0.17
	8.0	12	1444	201	0.14

*Coefficient of Variation

Candidate Organisms--

The eight candidate organisms selected for the initial work were the green Chlamydomonas, Chlorella, Chlorotyllium, and Ulothrix and the blue-green Gleotrichia, Nostoc 586, Nostoc 31 and Oscillatoria. Each alga was exposed to the nuclides for three hours in a dark environment.

Effect of Biomass--

The effect of biomass was studied by use of Nostoc 586 and ^{210}Pb. The cells were centrifuged, washed, and resuspended in various amounts of distilled water to achieve the desired relative concentration. Results of this experiment are given in Table 8.

TABLE 8. EFFECT OF BIOMASS ON LEAD 210 REMOVAL BY NOSTOC 586

Relative concentration of Nostoc 586	Dry wt. (mg/ml)	Residual CPM in supernatant*
0.24	0.21	4210 ± 520
0.5	0.43	3930 ± 500
1	0.86	2990 ± 720
2	1.72	2080 ± 220
4	3.44	2980 ± 660
8	6.88	1930 ± 170
Control	0	3240 ± 350

* Mean of 6 wells ± 1 standard deviation; control is mean of 10 wells.

TABLE 9. INFLUENCE OF CULTURE AGE ON REMOVAL OF LEAD 210

Organism	Age (days)	Dry wt. (mg/ml)	Residual CPM in supernatant*
Nostoc 586	15	0.71	2000 ± 520
	55	1.40	1790 ± 210
Chlamydomonas	15	0.15	1210 ± 180
	55	0.13	2270 ± 320
Oscillatoria	15	0.31	2600 ± 600
	55	0.71	1520 ± 130
Control	--	0	3340 ± 400

*Mean of 6 wells ± standard deviation.

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TABLE 10. REMOVAL OF ^{210}Pb and ^{109}Cd BY WATER SAMPLES* SEEDED WITH NOSTOC 586 and ULOTHRIX

		Residual CPM[††] in Supernatants			
		Nostoc 586		Ulothrix	
Water Source[†]	pH	^{210}Pb	^{109}Cd	^{210}Pb	^{109}Cd
1) Onondaga Lake	7.4	2430	25800	2370	24600
2) Nine Mile Creek (State Fair Blvd)	7.3	2860	29200	3100	27100
3) Nine Mile Creek (Amboy)	7.7	2740	21200	2380	20500
4) Geddes Brook	7.5	2570	24900	2570	21000
5) Meadowbrook Crk.	7.1	1840	21900	2640	23900
6) Allied effluent	7.6	2900	24000	2590	23700
1/10 Bristol's nitrate solution	6.8	2140	18100	1980	21600
Distilled H_2O	6.7	2310	20700	2050	22500
No inoculum		2530 ± 280			24400 ± 2600

* All samples 0.5 ppm Pb; 0.025 ppm Cd.

[†] Water Quality:

1) = Saline lake receiving metro Treatment Plant effluent,
2) = Sterile with chlorine, sodium carbonate, sodium chloride
3) = Rural farm stream, downstream from Camillus village,
4) = Suburban stream, surface runoff; trout, pan fish,
5) = Urban stream, storm overflow channel, and
6) = Unnamed brook contaminated with chemical plant cooling water.

[††] Average of 6 wells; control = average of 12 wells ± 1 standard deviation.

Influence of Culture Age--

The influence of culture age on lead removal was studied by comparing the uptake of lead 210 by 15-and 55-day old cultures of Nostoc 586, Chlamydomonas, and Oscillatoria. Results of this study are presented in Table 9.

Heavy Metal Accumulation from Natural Waters--

Samples from various sources of water were gathered for the experiment described in Table 10. Each sample was seeded with radionuclide (either ^{210}Pb or ^{109}Cd), algae (either Nostoc 586 or Ulothrix), and the uptake of the metal determined. The pH of the various water samples ranged from 7.1 to 7.7. Distilled water and the normal maintenance medium (Bristol's solution diluted 1/10) were also used to compare with uptake from the various natural water samples.

Improved Technique-Protocol II

General Discussion--

The results of the initial screenings, coupled with the increased capacity of the improved technique, led to the decision to screen for heavy metal removal over a broader range of pHs. Therefore, results were obtained using the buffers and pHs described in Table 11.

TABLE 11. BUFFERS* USED IN IMPROVED MICROTITER ASSAY (Protocol II)

pH	Component A	Component B
3	0.05M $KHC_8H_4O_4$	0.22M HCl
4	0.05M $KHC_8H_4O_4$	0.0001M HCl
5	0.05M $KHC_8H_4O_4$	0.023M NaOH
6	0.05M KH_2PO_4	0.0006M NaOH
7	0.05M KH_2PO_4	0.29M NaOH
8	0.05M KH_2PO_4	0.047M NaOH
9	0.013M $Na_2B_4O_7 \cdot 10H_2O$	0.005M HCl
10	0.13M $Na_2B_4O_7 \cdot 10H_2O$	0.02M NaOH

* Handbook of Chemistry and Physics, 57th Ed., 1977.

The radionuclides used in this part of the study were ^{109}Cd, ^{203}Hg, ^{210}Pb, and ^{65}Zn. Arsenic was excluded because of the negative results obtained during the first phase of the study and also because, with a half-life of only 26.8 hrs. the original activity rapidly diminished and required large corrections for decay within the experimental period.

Precision--

The precision of the new technique, as well as the necessity for a change in procedure, is demonstrated by the following data. A plate was prepared with 3 drops of water and 1 drop of ^{203}Hg solution, sampled, and the glass tubes counted immediately in the gamma counter. The same tubes were counted 18, 66, and 90 hours after the initial counts. The data obtained are presented in Table 12.

The striking downward trend of the mean counts led to the hypothesis that, as the liquid medium evaporated from the top of cellulose acetate cartridges, the radionuclide was redistributed along the length of the filter element, thereby changing the counting geometry and the mean counts. This hypothesis was tested in two ways. First, some dye was used to monitor visually any upward transport of dissolved matter as the solvent evaporated. It was observed that movement of the dye ceased after a period of about 3 days. Second, a plate was prepared and sampled and the tubes placed in an oven at 60°C for about 12 hours before counting. The tubes were recounted after 12 hours.

TABLE 12. MERCURY 203 COUNTS FOR A CONTROL PLATE

Time (hrs)	n*	$\bar{X}$	x	CV+
0	8	7253	628	0.087
18	8	6007	509	0.085
66	8	5162	338	0.065
90	8	5056	269	0.053

Notes: *Data from one column.
+Coefficient of variation.

Though the first counts (with data analyzed for the 12 wells of the first row) produced a mean of 5786, a standard deviation of 583, and a coefficient of variation of 0.10, the second series of counts of the same tubes produced a mean of 5749, a standard deviation of 567, and a coefficient of variation (again) of 0.10.

While the above observations certanly do not prove the hypothesis as stated, the goal of reproducible counts was obtained. All tubes were therefore dried at 60°C for at least 6 hours prior to counting.

Results--

The results from the screening experiment will be presented in tabular form. For this series of experiments all algae were exposed to the nuclide for 3 hours in the dark. Table 13 shows the algal cultures and ages used with the various nuclides.

TABLE 13. ALGAE USED IN IMPROVED MICROTITER ASSAY (PROTOCOL II)

Alga	Young Cultures*				Old Cultures†			
	^{109}Cd	^{210}Pb	^{203}Hg	^{65}Zn	^{109}Cd	^{210}Pb	^{203}Hg	^{65}Zn
Navicula pelliculosa	+	+	+	+	+	+		
Chlamydomonas	+	+	+	+		+		+
Chlorella	+	+	+	+				
Mougeotia	+	+	+	+				
Scenedesmus	+	+	+	+	+		+	
Ulothrix	+	+	+	+				
Zygnema	+	+	+	+				
Gleotrichia					+	+	+	+
Nostoc 586	+	+	+	+	+	+	+	
Nostoc muscorum A	+	+	+	+	+	+		+
Nostoc H	+	+	+	+	+	+	+	
Nostoc L						+		+
Nostoc W						+		+
Oscillatoria	+	+	+	+		+		
Schizothrix	+	+	+	+		+	+	
Cazenovia (Bristol's)	+	+	+	+				
Cazenovia (Diatom)	+	+	+	+				
Onondaga (Bristol's)	+	+	+	+				
Onondaga (Diatom)	+	+	+	+				

* Cultures were 11 days old
† Cultures were 44 days old

11-Day algae and cadmium--The objective of the statistical program was to identify those algal-pH combinations that revealed significant removals of cadmium. This was accomplished by identifying experimental means lower (at the 95% confidence level) than the corresponding control mean. Table 14 shows the analysis of variance data used to determine significant differences between experimental and control means, while Table 15 shows those algal-pH combinations that revealed significant removals of cadmium.

The F-test is used in a sample calculation to show how the data were used to determine significant removals. Figure 3 (page 35) depicts the experimental design used in these assays. The interplate control (IPC) data were used to test the hypothesis that all experimental plates were handled similarly to the control plate. The calculation uses the treatment mean square (TMS) and the error mean square (EMS) to calculate an F value. Using the IPC (pH 3) data from Table 14 gives:

TABLE 14. STATISTICAL DATA FOR CADMIUM - YOUNG ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	28.9	55.0	34.4	57.0	127	69.5	52.3	75.5
Treatment df[†]	18	18	18	18	18	18	18	18
Treatment MS*	1.60	3.06	1.91	3.17	7.04	3.86	2.91	4.19
Error SS	1.59	192	133	88.0	119	89.3	139	136
Error df	101	101	101	101	101	101	101	101
Error MS	1.57	1.90	1.32	0.871	1.18	0.884	1.38	1.35
Total SS	188	247	168	145	246	159	191	212
Total df	119	119	119	119	119	119	119	119
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	8960	7570	7960	7530	6540	6080	5640	7380
σ_x	1130	890	660	830	870	930	1090	1530

---

*All sums of squares (SS) and mean squares (MS) are x 10^{-6}.

†Degrees of freedom.

TABLE 15. SIGNIFICANT REMOVALS OF CADMIUM* BY YOUNG CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Navicula pelliculosa (0.085)							
t-value	ns[†]	ns	4.99[††]	4.47	5.38	ns	ns
Mean			5200	4110	3550		
σ_x			830	380	1050		
Removal efficiency			0.31	0.37	0.42		
Accumulation coefficient			1330	1600	1790		
Concentration factor			3640	4370	4900		
Chlamydomonas (0.04)							
t-value	ns	ns	3.54	ns	ns	ns	ns
Mean			5880				
σ_x			730				
Removal efficiency			0.22				
Accumulation coefficient			2010				
Concentration factor			5480				
Chlorella pyrenoidosa (0.015)							
t-value	ns	3.78	4.04	5.78	3.84	ns	3.28
Mean		5790	5640	3400	4270		5490
σ_x		810	1020	510	550		600
Removal efficiency		0.27	0.25	0.48	0.30		0.26
Accumulation coefficient		6660	6100	11,700	7520		6250
Concentration factor		18,200	16,700	32,000	19,800		17,100

(continued)

TABLE 15. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Scenedesmus obliquus							
t-value	ns	ns	3.70	3.69	3.40	ns	ns
Mean			5800	4540	4480		
σ_x			780	610	670		
Removal efficiency			0.23	0.31	0.26		
Accumulation coefficient			990	1320	1130		
Concentration factor			2700	3600	3100		
Ulothrix fimbrinata (0.017)							
t-value	ns	3.33	3.13	ns	ns	ns	ns
Mean		6050	6070				
σ_x		890	790				
Removal efficiency		0.24	0.19				
Accumulation coefficient		5170	4180				
Concentration factor		14,100	11,400				
Zygnema (0.03)							
t-value	ns	ns	4.14	ns	ns	ns	ns
Mean	5590						
σ_x	670						
Removal efficiency	0.26						
Accumulation coefficient	3130						
Concentration factor	8560						

(continued)

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TABLE 15. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc 586 (0.17)							
t-value	ns	2.977	3.12	ns	3.22	ns	ns
Mean		6250	6070		4560		
σ_x		990	820		730		
Removal efficiency		0.21	0.19		0.25		
Accumulation coefficient		460	420		540		
Concentration factor		1260	1140		1470		
Oscillatoria (0.19)							
t-value	ns	ns	3.55	3.00	ns	ns	ns
Mean			5870	4910			
σ_x			1270	460			
Removal efficiency			0.22	0.25			
Accumulation coefficient			420	480			
Concentration factor			1160	1310			
Onondaga - Bristol's (0.23)							
t-value	ns	ns	ns	3.17	ns	ns	ns
Mean				4820			
σ_x				760			
Removal efficiency				0.26			
Accumulation coefficient				420			
Concentration factor				1140			

(continued)

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TABLE 15. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Onondaga - Diatom (0.175)							
t-value	ns	ns	ns	ns	2.96	ns	ns
Mean					4680		
σ_x					410		
Removal efficiency					0.23		
Accumulation coefficient					480		
Concentration factor					1310		

---

[*] Cadmium concentration = 0.36 μg/ml; activity = 1.24 μC/ml.

[†] Not significant

[††] t_{crit} = 2.90 at the 95% confidence level.

$$\frac{1.60 \quad \text{(TMS)}}{1.57 \quad \text{(EMS)}} = 1.02$$

This value is to be compared with a tabulated F value (Steel and Torrie, 1960, p. 436-441) having 18 numerator and 101 denominator degrees of freedom (treatment and error degrees of freedom respectively). The interpolated value is 1.50. Since the calculated F value is lower than the tabulated F value, the hypothesis that all plates were treated similarly is accepted. A calculated F value higher from the tabulated value would indicate that at least one IPC mean was different from the other control means and would be evidence of some systematic error. The data in the following tables showed no evidence of systematic error.

The next step is to identify specific algal-pH combinations revealing significant cadmium removals. This was done by use of Dunnett's _t_ criterion. The calculation uses a control and experimental mean at a given pH, the error mean square (EMS) for that pH, and the number of experimental and control replicates. Using _Navicula pelliculosa_ at pH 6 for an example, one finds the experimental mean of 5200 (Table 15), control mean and EMS of 7530 and 8.7×10^5, respectively (Table 14) and 6 and 12 experimental and control replicates, respectively (Figure 3).

The calculated _t_ - value is therefore

$$t = \frac{7530 \quad - \quad 5200}{\sqrt{8.71 \times 10^5 \; (1/12 + 1/6)}} = 4.99$$

The calculated _t_ value is compared with tabulated values of Dunnett's _t_ (Steel and Torrie, 1960, p.447) using 18 treatment means and 101 error degrees of freedom. The extrapolated value is 2.90. The larger calculated _t_ value indicates this experimental mean is significantly different from the pH 6 control mean and is taken as evidence of a significant removal of cadmium by _Navicula pelliculosa_ at this pH.

The accumulation coefficient (μg metal accumulated per gram of algal dry weight) can be calculated from the data in Table 15. Again using the data from _Navicula pelliculosa_ at pH 6, the calculation is:

$$\frac{0.31 \times 0.366 \ \mu g/ml}{0.085 \ mg/ml \times 1g/1000 \ mg}$$

This assumes that all the metal removed is concentrated by the alga. Since 80 to 85% of the supernatant is collected by the cartridges, most of the remaining volume is the algal cells. The assumption is therefore reasonable.

The concentration factor is simply the accumulation coefficient divided by the initial metal concentration. For the same data, this is:

$$\frac{1330 \ \mu g/g}{0.366 \ \mu g/ml} = 3630$$

Slight discrepancies (such as the tabular value of 3640 versus 3630 calculated here) are caused by rounding errors. The data in the tables were calculated and then rounded to 3 significant digits but the calculations used values already rounded.

An examination of the data in Table 15 reveals some interesting trends. First, the most efficient accumulator of metal is clearly *Chlorella pyrenoidosa*, with concentration factors on the order of 10^4 over the range of pHs from 5 to 8. *Ulothrix fimbrinata* also shows high concentration factors but only at pH 5 and 6. In general, the blue-green algae were not efficient accumulators of cadmium.

11-Day algae and lead--The experimental results for young algae and lead are presented in Tables 16 and 17. The most noteworthy feature of these results is the dramatic superiority of *Chlamydomonas* to accumulate lead. Between 70 and 80% of the lead was removed at each pH from 4 to 9. Concentration factors were on the order of 10^4. No other algae removed as much lead over as wide a pH range.

11-Day algae and mercury--Tables 18 and 19 show the results of the young algae-mercury experiment. The highest percentage of removals were achieved by the blue-green algae, especially *Nostoc* H and *Nostoc* 586. The acidic pHs favored removal of mercury by these organisms. The highest concentration factors were exhibited by *Chlorella pyrenoidosa*, with values on the order of 10^4 for all pHs from 4 to 10. The mixed cultures of algae (i.e., Cazenovia and Onondaga Lake algae) removed mercury from solution but not as efficiently as the most successful pure cultures.

11-Day algae and zinc--The data for the zinc-young algae experiment are shown in Table 20. The application of the F-test to the data at each pH shows that at least one mean was significantly different from the others at pHs 7 to 10. This is in fact the case in as much as the means for *Mougeotia* at these pHs were significantly higher than the control mean. Assuming a systematic error and re-analyzing the data without the *Mougeotia* data, one would reach the conclusion that no organism removed zinc to a statistically significant degree under the conditions employed in this study. The lower counts on the control plate at pH 9 and 10 were undoubtedly due to a pH effect because they were duplicated on the other two control plates.

44-Day algae and cadmium--Tables 21 and 22 show the data from the cadmium-old algae experiment. As in the earlier experiment with young algae and cadmium, no single organism is superior to any other. In fact, removals are no better than 30% (for *Navicula pelliculosa* at pH 8). In general, it appears that an acidic pH favors cadmium accumulation by algae. As in all experiments with older cultures of algae, the accumulation coefficients and concentration factors are dramatically lower than younger cultures of the same species.

44-Day algae and lead--Data from the lead-old algae experiment are presented in Tables 23 and 24. As in the young algae lead experiment, the single organism that accumulates lead most effectively is *Chlamydomonas*, which removed between 60 and 70% of the lead at pH 4 to 9. As in the young culture experiment, there was no significant removal at pH 10. Concentration factors were on the order of 10^2. The next most competent accumulators of lead were the blue-green *Nostoc* H and *Nostoc* W which removed between 30 to 40% and 10 to 20% of the lead, respectively. Concentration factors again were on the order of 10^2. Removal was fairly consistent from pH 4 to 9.

TABLE 16. STATISTICAL DATA FOR LEAD - YOUNG ALGAE EXPERIMENTS

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	7.40	41.8	35.9	38.7	38.5	36.1	52.9	21.8
Treatment df	18	18	18	18	18	18	18	18
Treatment MS*	0.411	2.33	1.99	2.15	2.14	2.00	1.94	1.21
Error SS	34.5	55.1	48.2	27.2	29.8	33.9	21.2	32.8
Error df	101	101	101	101	101	101	101	101
Error MS	0.344	0.546	0.477	0.269	0.295	0.335	0.210	0.325
Total SS	42.2	97.0	84.0	65.9	68.3	69.9	74.1	54.7
Total df	119	119	119	119	119	119	119	119
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	3780	3010	3100	3210	3390	3100	3260	3280
σ_x	520	540	390	500	730	620	380	330

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*All sums of squares (SS) and mean squares (MS) are x 10^{-6}.

†Degrees of freedom.

TABLE 17. SIGNIFICANT REMOVALS OF LEAD* BY YOUNG CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Chlamydomonas (0.04)							
t-value	6.46††	6.40	9.12	8.45	7.12	11.0	ns††
Mean	670	880	830	1130	950	640	
σ_x	190	200	112	190	130		
Removal efficiency	0.78	0.72	0.74	0.69	0.69	0.8[illegible]	
Accumulation coefficient	490	450	460	420	430	500	
Concentration factor	19,500	17,900	18,600	16,700	17,400	20,100	
Mougeotia (0.035)							
t-value	ns††	ns	ns	ns	ns	3.93	ns
Mean						2320	
σ_x						1020	
Removal efficiency						0.29	
Accumulation coefficient						210	
Concentration factor						8180	
Ulothrix fimbrinata (0.017)							
t-value	ns	ns	ns	ns		4.65	
Mean						2150	
σ_x						350	
Removal efficiency						0.34	
Accumulation coefficient						500	
Concentration factor						19,900	

(continued)

TABLE 17. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc muscorum A (0.005)							
t-value	ns	ns	ns	ns	ns	3.01	ns
Mean						2540	
σ_x						470	
Removal efficiency						0.22	
Accumulation coefficient						1090	
Concentration factor						43,800	
Nostoc 586 (0.017)							
t-value	ns	ns	3.26	4.13	ns	5.64	ns
Mean			2360	2290		1920	
σ_x			1400	200		330	
Removal efficiency			0.27	0.33		0.41	
Accumulation coefficient			39	48		60	
Concentration factor			1560	1920		2400	
Nostoc H (0.23)							
t-value	ns	ns	4.48	ns	ns	4.80	ns
Mean			2040			2120	
σ_x			200			370	
Removal efficiency			0.36			0.35	
Accumulation coefficient			40			38	
Concentration factor			1580			1520	

(continued)

TABLE 17. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Oscillatoria (0.19)							
t-value	ns	ns	ns	ns	ns	4.10	ns
Mean						2320	
σ_x						230	
Removal efficiency						0.29	
Accumulation coefficient						38	
Concentration factor						1520	
Schizothrix calcicola (0.23)							
t-value	ns	ns	ns	3.91	ns	4.00	4.32
Mean				2340		2310	2060
σ_x				210		220	310
Removal efficiency				0.31		0.24	0.37
Accumulation coefficient				35		33	42
Concentration factor				1410		1320	1690

[*] Lead concentration 0.025 µg/ml; 1.67 µC/ml.

[†] t_{crit} = 2.90 at 95% confidence level.

[††] Not significant.

TABLE 18. STATISTICAL DATA FOR MERCURY - YOUNG ALGAE EXPERIMENTS

	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	43.1	122	71.1	87.0	33.1	36.9	40.1	101
Treatment df†	18	18	18	18	18	18	18	18
Treatment MS *	2.39	6.79	3.95	4.83	1.84	2.05	2.23	5.60
Error SS	136	54.4	36.7	45.9	13.5	34.7	30.0	48.5
Error df	101	101	101	101	101	101	101	101
Error MS	1.35	0.539	0.363	0.454	0.134	0.344	0.297	0.480
Total SS	179	176	107	133	46.6	71.7	70.1	149
Total df	119	119	119	119	119	119	119	119
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	5900	4000	3080	4150	2980	2680	2880	3530
σ_x	830	1230	1160	750	250	580	410	530

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*All sums of squares (SS) and mean squares (MS) are x 10^{-6}.

†Degrees of freedom.

TABLE 19. SIGNIFICANT REMOVALS OF MERCURY* BY YOUNG CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Navicula pelliculosa (0.085)							
t-value	4.37[†]	3.73	7.09	4.79	3.15	4.87	3.57
Mean	2400	1950	1760	2100	1760	1550	2290
σ_x	480	430	410	300	220	210	250
Removal efficiency	0.40	0.37	0.58	0.29	0.34	0.46	0.35
Accumulation coefficient	190	180	280	140	170	220	170
Concentration factor	4700	4290	6780	3460	4040	5420	4130
Chlamydomonas (0.04)							
t-value	6.90	4.39	4.46	ns[††]	ns	4.63	ns
Mean	1470	1750	2640			1620	
σ_x	210	870	310			420	
Removal efficiency	0.63	0.43	0.36			0.44	
Accumulation coefficient	650	440	370			450	
Concentration factor	15,810	10,700	9060			10,900	
Chlorella pyrenoidosa (0.015)							
t-value	5.74	3.67	8.15	11.5	6.50	7.10	4.85
Mean	1900	1970	1400	890	780	950	1850
σ_x	260	230	220	110	210	130	340
Removal efficiency	0.53	0.36	0.66	0.70	0.71	0.67	0.48
Accumulation coefficient	1440	980	1810	1920	1940	1830	1300
Concentration factor	35,100	24,000	44,100	46,700	47,300	44,800	31,700

(continued)

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TABLE 19. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Mougeotia (0.035)							
t-value	ns	ns	5.53	6.47	ns	4.06	4.14
Mean			2280	1790		1780	2090
σ_x			270	240		430	360
Removal efficiency			0.45	0.40		0.38	0.41
Accumulation coefficient			530	470		450	480
Concentration factor							
Scenedesmus obliquus (0.085)							
t-value	ns	3.07	6.84	6.97	4.05	5.70	4.47
Mean		2150	1840	1700	1500	1330	1980
σ_x		420	290	180	340	250	230
Removal efficiency		0.30	0.56	0.43	0.44	0.54	0.44
Accumulation coefficient		150	270	210	210	260	210
Concentration factor		3540	6540	5040	5190	6340	5160
Ulothrix fimbrinata (0.017)							
t-value	4.77	ns	3.81	ns	ns	3.79	4.18
Mean	2250		2860			1850	2080
σ_x	730		600			550	260
Removal efficiency	0.44		0.31			0.36	0.41
Accumulation coefficient	1060		750			860	990
Concentration factor	25,740		18,220			21,070	24,130

(continued)

TABLE 19. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Zygnema (0.030)							
t-value	3.34	ns	6.31	4.50	3.29	3.74	3.45
Mean	2780		2020	2150	1720	1860	2330
σ_x	560		210	370	270	350	500
Removal efficiency	0.31		0.51	0.28	0.36	0.35	0.34
Accumulation coefficient	420		700	380	490	480	460
Concentration factor	10,200		17,100	9,220	12,000	11,800	11,300
Nostoc 586 (0.17)							
t-value	9.50	8.12	8.64	5.41	5.14	7.34	5.67
Mean	520	630	1240	1970	1180	880	1570
σ_x	130	170	790	360	550	390	760
Removal efficiency	0.87	0.80	0.70	0.33	0.56	0.69	0.56
Accumulation coefficient	210	190	170	80	140	170	130
Concentration factor	5120	4680	4130	1950	3290	4080	3270
Nostoc muscorum A (0.005)							
t-value	ns	ns	4.18	3.54	3.06	4.62	3.41
Mean			2740	2330	1790	1620	2350
σ_x			490	320	220	420	250
Removal efficiency			0.34	0.22	0.33	0.44	0.33
Accumulation coefficient			2790	1780	2740	3590	2740
Concentration factor			68,000	43,500	66,900	87,500	66,900

(continued)

TABLE 19. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc H (0.23)							
t-value	8.36	7.19	7.43	6.81	3.31	5.46	4.14
Mean	940	910	1640	1730	1720	1390	2100
σ_x	90	60	190	200	730	1120	620
Removal efficiency	0.77	0.70	0.60	0.42	0.36	0.52	0.41
Accumulation coefficient	140	130	110	70	60	90	70
Concentration factor	3330	3060	2630	1820	1570	2240	1770
Oscillatoria (0.19)							
t-value	7.01	6.63	7.88	ns	3.93	5.75	ns
Mean	1430	1080	1490		1530	1310	
σ_x	460	620	670		890	810	
Removal efficiency	0.64	0.65	0.64		0.43	0.54	
Accumulation coefficient	140	140	140		90	120	
Concentration factor	3380	3420	3370		2260	2860	
Schizothrix calcicola (0.22)							
t-value	6.21	6.19	6.47	7.69	6.12	7.15	6.29
Mean	1720	1210	1970	1570	890	930	1350
σ_x	520	180	200	150	130	30	370
Removal efficiency	0.57	0.61	0.53	0.47	0.67	0.68	0.62
Accumulation coefficient	110	110	100	90	120	130	110
Concentration factor	2590	2760	2390	2150	3040	3070	2800

(continued)

TABLE 19. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Cazenovia - Bristol's (0.25)							
t-value	ns	ns	6.82	3.94	4.16	4.84	ns
Mean			1850	2260	1470	1560	
σ_x			280	500	310	250	
Removal efficiency			0.55	0.24	0.45	0.46	
Accumulation coefficient			90	40	70	80	
Concentration factor			2220	970	1820	1830	
Cazenovia - Diatom (0.005)							
t-value	ns	ns	8.22	5.86	4.20	5.23	3.66
Mean			1380	1900	1460	1370	2260
σ_x			180	330	130	240	330
Removal efficiency			0.69	0.36	0.46	0.52	0.36
Accumulation coefficient			5480	2950	3760	4290	2950
Concentration factor			13,000	71,900	91,600	105,000	71,800
Onondaga - Bristol's (0.23)							
t-value	4.20	3.35	3.83	5.46	ns	ns	ns
Mean	2460	2060	2850	1980			
σ_x	430	420	2170	210			
Removal efficiency	0.38	0.33	0.31	0.33			
Accumulation coefficient	70	60	60	60			
Concentration factor	1670	1430	1350	1460			

(continued)

TABLE 19. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Onondaga - Diatom (0.175)							
t-value	4.24	4.24	8.94	7.90	5.16	5.87	4.72
Mean	2450	1800	1130	1530	1170	1280	1895
σ_x	630	500	200	320	250	270	320
Removal efficiency	0.39	0.42	0.73	0.49	0.56	0.56	0.46
Accumulation coefficient	90	100	170	110	130	130	110
Concentration factor	2220	2370	4150	2770	3220	3170	2640

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[*] Mercury concentration 0.041 μg/ml; 0.243 μC/ml.

[†] t_{crit} = 2.90 at 95% confidence level.

[±] Not significant.

TABLE 20. STATISTICAL DATA FOR ZINC - YOUNG ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	5.11	7.70	6.41	6.72	56.7	44.7	25.0	18.9
Treatment df†	18	18	18	18	18	18	18	18
Treatment MS*	0.284	0.428	0.356	0.373	3.15	2.48	1.39	1.05
Error SS	38.8	39.0	28.8	48.2	47.1	77.4	34.9	25.6
Error df	101	101	101	101	101	101	101	101
Error MS	0.384	0.386	0.285	0.478	0.466	0.766	0.345	0.253
Total SS	43.9	46.7	35.2	55.0	104	122	59.9	44.4
Total df	119	119	119	119	119	119	119	119
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	4500	3680	3710	3660	2250	2200	1190	560
σ_x	320	740	780	560	750	750	690	210

* All sums of squares (SS) and mean squares (MS) are x 10^{-6}.

† Degrees of freedom.

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TABLE 21. STATISTICAL DATA FOR CADMIUM - OLD ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	16.7	66.7	48.6	390	209	284	897	552
Treatment df†	8	8	8	8	8	8	8	8
Treatment MS*	2.09	8.33	6.08	48.7	26.1	35.5	112	69.1
Error SS	167	59.0	56.0	363	509	473	471	505
Error df	51	51	51	51	51	51	51	51
Error MS	3.27	1.16	1.10	7.13	9.98	9.28	9.24	9.90
Total SS	183	126	105	753	718	757	1369	1057
Total df	59	59	59	59	59	59	59	59
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	7020	7010	7140	7570	5190	4570	4290	4860
σ_x	330	230	300	500	780	810	930	1060

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*All sums of squares (SS) and mean squares (MS) are x 10^{-5}.

†Degrees of freedom.

TABLE 22. SIGNIFICANT REMOVALS OF CADMIUM* BY OLD CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Navicula pelliculosa (0.95)							
t-value	ns††	2.88†	3.86	ns	2.89	ns	ns
Mean		6670	5940		3190		
σ_x		360	510		660		
Removal efficiency		0.07	0.21		0.30		
Accumulation coefficient		17	54		80		
Concentration factor		70	230		320		
Scenedesmus obliquus (1.88)							
t-value	2.91	ns	3.56	ns	ns	ns	ns
Mean	6510		6070				
σ_x	70		150				
Removal efficiency	0.07		0.20				
Accumulation coefficient	9		25				
Concentration factor	40		110				
Gleotrichia (1.80)							
t-value	6.57	5.53	5.54	ns	ns	ns	ns
Mean	5890	6230	5450				
σ_x	250	310	640				
Removal efficiency	0.16	0.13	0.28				
Accumulation coefficient	21	17	37				
Concentration factor	90	70	160				

(continued)

TABLE 22. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc 586 (2.03)							
t-value	ns	4.19	3.37	ns	ns	ns	ns
Mean		6450	6150				
σ_x		210	330				
Removal efficiency		0.10	0.19				
Accumulation coefficient		11	22				
Concentration factor		50	90				
Nostoc muscorum A (1.88)							
t-value	4.47	4.15	ns	ns	ns	ns	ns
Mean	6250	6450					
σ_x	350	250					
Removal efficiency	0.11	0.10					
Accumulation coefficient	14	12					
Concentration factor	60	50					
Nostoc H (1.76)							
t-value	4.39	2.89	3.97	ns	ns	ns	ns
Mean	6260	6660	5900				
σ_x	300	510	350				
Removal efficiency	0.11	0.07	0.22				
Accumulation coefficient	14	9	30				
Concentration factor	60	40	130				

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* Cadmium concentration 0.238 μg/ml; 0.802 μC/ml.

† t_{crit} = 2.87 at 95% confidence level.

‡ Not significant

TABLE 23. STATISTICAL DATA FOR LEAD - OLD ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	1.16	134	101	138	101	99.6	114	18.9
Treatment df†	10	10	10	10	10	10	10	10
Treatment MS*	0.116	13.4	10.1	13.8	10.1	9.96	11.4	1.89
Error SS	14.8	8.17	8.13	7.75	9.70	11.0	7.58	13.0
Error df	61	61	61	61	61	61	61	61
Error MS	0.243	0.134	0.133	0.127	0.159	0.181	0.124	0.213
Total SS	16.0	143	109	146	111	111	122	31.9
Total df	71	71	71	71	71	71	71	71
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	6470	6290	6290	6560	6660	6680	6380	5080
σ_x	790	280	340	470	310	310	380	270

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*All sums of squares (SS) and mean squares (MS) are x 10^{-6}.

†Degrees of freedom.

TABLE 24. SIGNIFICANT REMOVALS OF LEAD* BY OLD CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Chlamydomonas (1.96)							
t-value	23.9†	20.4	26.0	19.4	18.5	23.9	ns††
Mean	1910	2570	1930	2780	2750	2170	
σ_x	210	400	110	380	250	120	
Removal efficiency	0.70	0.59	0.71	0.58	0.59	0.66	
Accumulation coefficient	13.0	11.0	13.0	11.0	11.0	13.0	
Concentration factor	350	300	360	300	300	340	
Gleotrichia (1.80)							
t-value	8.94	8.40	9.61	ns	ns	3.79	ns
Mean	4660	4680	4850			5710	
σ_x	450	430	390			760	
Removal efficiency	0.26	0.26	0.26			0.10	
Accumulation coefficient	5.40	5.20	5.40			2.20	
Concentration factor	140	140	150			58.0	
Nostoc 586 (2.03)							
t-value	4.02	ns	6.83	5.79	3.88	8.46	
Mean	5560		5340	5510	5860	4890	
σ_x	360		280	150	320	190	
Removal efficiency	0.12		0.19	0.17	0.12	0.23	
Accumulation coefficient	2.20		3.40	3.20	2.30	4.30	
Concentration factor	60		90	90	60	120	

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TABLE 24. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc H (1.76)							
t-value	10.7	8.50	14.6	11.1	8.61	13.9	ns
Mean	4340	4740	3950	4450	4850	3930	
σ_x	500	500	210	320	270	310	
Removal efficiency	0.31	0.25	0.40	0.33	0.27	0.38	
Accumulation coefficient	6.6	5.3	8.50	7.00	5.80	8.20	
Concentration factor	180	140	230	190	160	220	
Nostoc L (1.56)							
t-value	2.92	ns	ns	ns	ns	3.63	ns
Mean	5760					5740	
σ_x	260					200	
Removal efficiency	0.09					0.10	
Accumulation coefficient	2.00					2.40	
Concentration factor	50					60	
Nostoc W (1.29)							
t-value	5.20	5.61	6.65	5.68	ns	7.00	ns
Mean	5340	5270	5370	5530		5150	
σ_x	740	420	220	250		390	
Removal efficiency	0.15	0.16	0.18	0.17		0.19	
Accumulation coefficient	4.40	4.70	5.30	4.90		5.60	
Concentration factor	120	130	140	130		150	

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TABLE 24. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Schizothrix calcicola **(1.95)**							
t-value	3.83	ns	6.52	5.39	3.63	4.94	ns
Mean	5590		5400	5590	5910	5510	
σ_x	210		200	360	160	210	
Removal efficiency	0.11		0.18	0.16	0.12	0.14	
Accumulation coefficient	2.10		3.40	3.10	2.20	2.60	
Concentration factor	60		90	80	60	70	
Oscillatoria **(1.73)**							
t-value	15.6	4.41	12.2	5.45	5.91	12.0	ns
Mean	3440	5490	4390	5570	5430	4260	
σ_x	170	350	320	590	460	320	
Removal efficiency	0.45	0.13	0.33	0.16	0.19	0.37	
Accumulation coefficient	9.80	2.80	7.10	3.50	4.10	7.20	
Concentration factor	260	70	190	90	110	190	

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* Lead concentration 0.0375 μg/ml; 0.25 μC/ml.

† t_{crit} = 2.89 at 95% confidence level.

†† Not significant.

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TABLE 25. STATISTICAL DATA FOR MERCURY - OLD ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS *	5.85	139	88.1	51.1	29.1	18.2	161	171
Treatment df †	5	5	5	5	5	5	5	5
Treatment MS *	1.17	27.8	17.6	10.2	5.82	3.64	32.2	34.2
Error SS	14.6	2.74	3.02	6.36	14.9	4.67	3.63	10.4
Error df	30	30	30	30	30	30	30	30
Error MS	0.487	0.0913	1.01	2.12	0.499	0.156	0.121	0.347
Total SS	20.5	142	91.1	57.4	44.1	22.9	165	181
Total df	35	35	35	35	35	35	35	35
Control data								
n	6	6	6	6	6	6	6	6
$\bar{X}$	3080	2440	1870	1650	1440	1190	1450	1580
$\sigma_{\bar{x}}$	110	110	90	80	130	180	140	160

* All sums of squares (SS) and mean squares (MS) are x 10^{-5}.

† Degrees of freedom.

44-Day algae and mercury--All five of the organisms screened in the mercury-old algae experiments showed significant removals of metal, as revealed by the data in Tables 25 and 26. Indeed, all the algae showed remarkably similar concentration factors (on the order of 10^2) and removal efficiencies (generally 60% to 70%). Once again, as in the young culture experiments, the acidic pHs favored mercury removal by the algae.

44-Day algae and zinc--The data for the zinc-old algae experiments are presented in Table 27. F-tests reveal significant effects at pH 9 and 10. However, as in the earlier experiments with zinc, the effects are probably related to changes in pH rather than to actual metal accumulation.. The raw data at pH 10 reveal that most treatment means are significantly higher than the control means, indicating that the experimental wells with algae apparently prevented the formation of insoluble zinc compounds like those that occurred in the control wells at pH 10.

Results from other Microtiter Assays (Protocol II)

Experiments Using Various Buffers at pH 6--

The ease with which data could be obtained with the new technique (Protocol II) led to the design of several experiments to answer more specific questions raised by the earlier results. One such experiment concerned the choice of buffers.

Chlamydomonas was chosen as the test organism and lead as the test metal. Several buffers, all at pH 6, were made up as described in Table 28. For this experiment, plates were made up in the normal manner with each group of 6 experimental wells having 6 control wells with the same buffer. Samples were obtained after the cells had been exposed to the buffer-metal combination for both 3 and 6 hours. A row of wells at pH 3 and without algae was utilized as an interplate control (IPC) as in previous experiments.

The results of this experiment (Table 28) show that the choice of buffer can affect the accumulation of lead by Chlamydomonas. One buffer, the citric acid-sodium citrate buffer, effectively prevented the uptake of lead by Chlamydomonas, probably because of the formation of a soluble complex. Citrate is well known as a chelating agent for certain metals (e.g. iron) and is often added to culture media to make the metallic micronutrients available for the organisms (Nichols 1973).

Except for the citrate buffer, all other buffers showed surprisingly similar results of removal efficiencies, accumulation coefficients, and concentration factors.

Experiments Over a 24-Hour Period

The improved technique (Protocol II) was used to gather data on metal accumulation by algae over a longer period of time. Algae found to accumulate metals were selected from the data of the screening experiments and representative pHs chosen. Plates were prepared in two duplicate 48-well (4 row by 12 column) sections, with each section having 5 groups of 6 experimental wells, two algae at each of two pHs, one alga at a third pH, and 3 rows of 6 control wells (one row for each pH). Four plates were prepared for each radionuclide used in this

TABLE 26. SIGNIFICANT REMOVALS OF MERCURY BY OLD CULTURES OF ALGAE

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Scenedesmus obliquus (1.88)							
t-value	22.1†	19.3	11.8	5.95	8.11	10.9	9.54
Mean	1210	750	660	670	600	750	550
σ_x	100	140	150	80	110	150	60
Removal efficiency	0.50	0.60	0.60	0.53	0.49	0.48	0.65
Accumulation coefficient	3.84	4.58	4.60	4.10	3.80	3.70	5.00
Concentration factor	270	320	320	280	260	250	350
Gleotrichia (1.80)							
t-value	35.3	22.7	9.49	2.77	5.39	ns‡	ns
Mean	490	560	1080	1080	800		
σ_x	70	130	180	520	210		
Removal efficiency	0.80	0.70	0.48	0.25	0.33		
Accumulation coefficient	6.40	5.60	3.90	2.00	2.60		
Concentration factor	440	390	270	140	180		
Nostoc 586 (2.03)							
t-value	24.7	23.7	12.7	5.89	8.52	ns	4.23
Mean	1070	500	590	680	580		1120
σ_x	90	50	240	20	40		300
Removal efficiency	0.56	0.73	0.65	0.53	0.51		0.29
Accumulation coefficient	4.00	5.20	4.60	3.80	3.70		2.10
Concentration factor	280	360	320	260	250		140

(continued)

TABLE 26. (continued)

ALGA (biomass mg/ml)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Nostoc H (1.76)							
t-value	27.1	23.4	11.5	4.60	6.28	ns	3.91
Mean	940	520	680	840	740		1150
	80	80	50	70	50		80
Removal efficiency	0.61	0.72	0.59	0.41	0.38		0.27
Accumulation coefficient	5.00	5.90	4.80	3.40	3.10		2.20
Concentration factor	350	410	330	240	220		150
Schizothrix calcicola (1.96)							
t-value	30.9	23.6	12.8	6.05	9.41	14.5	8.39
Mean	730	510	580	600	510	530	670
	120	70	60	30	70	40	120
Removal efficiency	0.70	0.78	0.65	0.54	0.57	0.64	0.57
Accumulation coefficient	5.20	5.40	4.80	4.00	4.20	4.70	4.20
Concentration factor	360	370	330	280	290	330	290

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* Mercury concentration = 0.0144 µg/ml; activity = 0.086 µC/ml.

† T_{crit} = 2.72 at the 95% confidence level.

‡ Not significant.

TABLE 27. STATISTICAL DATA FOR ZINC - OLD ALGAE EXPERIMENT

Source of Variability	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Treatment SS*	9.96	16.4	12.8	29.9	36.5	12.2	114	366
Treatment df†	8	8	8	8	8	8	8	8
Treatment MS*	1.24	2.05	1.60	3.74	4.56	1.53	14.2	45.8
Error SS	104	69.1	67.9	94.0	121	73.6	296	176
Error df	51	51	51	51	51	51	51	51
Error MS	2.04	1.35	1.33	1.84	2.37	1.44	5.80	3.46
Total SS	114	85.5	80.7	124	158	85.8	410	543
Total df	59	59	59	59	59	59	59	59
Control data								
n	12	12	12	12	12	12	12	12
$\bar{X}$	6410	6220	6350	6410	6760	6520	6460	720
σ_x	510	360	380	360	530	190	340	540

[*] All sums of squares (SS) and mean squares (MS) are x 10^{-5}.

[†] Degrees of freedom.

TABLE 28. RESULTS* OF BUFFER COMPARISON EXPERIMENT (pH 6) USING CHLAMYDOMONAS† AND LEAD 210††

Buffer	Time 3 hr	 6 hr
pH 6 Borax-phosphate (Borax and potassium dihydrogen phosphate)§		
Control mean	24,830	24,300
Control σ_x	1030	850
Experimental mean	11,500	10,400
Experimental σ_x	520	230
Removal efficiency	0.54	0.57
Accumulation coefficient	115	123
Concentration factor	1920	2040
pH 6 Citrate (sodium citrate and citric acid)§		
Control mean	24,800	24,000
Control σ_x	1270	600
Experimental mean	22,900	22,400
Experimental σ_x	1970	1660
Removal efficiency	0.08	0.07
Accumulation coefficient	16	14
Concentration factor	270	240
pH 6 Succinate (succinic acid and sodium hydroxide)§		
Control mean	23,600	22,600
Control σ_x	1060	960
Experimental mean	10,500	9600
Experimental σ_x	200	470
Removal efficiency	0.56	0.58
Accumulation coefficient	119	123
Concentration factor	1980	2050
pH 6 Citrate-phosphate (citric acid and sodium monohydrogen phosphate)§		
Control mean	23,260	22,600
Control σ_x	800	960
Experimental mean	12,100	9600
Experimental σ_x	500	470
Removal efficiency	0.48	0.58
Accumulation coefficient	103	123
Concentration factor	1710	2050

(continued)

TABLE 28. (continued)

Buffer	Time 3 hr	 6 hr
pH 6 Phthalate (potassium hydrogen phthalate and sodium hydroxide)[§]		
Control mean	21,700	21,300
Control σ_x	700	570
Experimental mean	10,500	9,600
Experimental σ_x	350	230
Removal efficient	0.52	0.55
Accumulation coefficient	110	118
Concentration factor	1840	1960
pH 6 Tris-maleate (tris, maleic anhydride and sodium hydroxide)[§]		
Control mean	21,700	20,200
Control σ_x	700	800
Experimental mean	9,400	7,700
Experimental σ_x	390	220
Removal efficiency	0.57	0.62
Accumulation coefficient	121	133
Concentration factor	2,020	2,210

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[*] For sample calculations see Section II. B. 2. of Chapter 4.

[†] Twelve day culture of algae; dry weight = 0.28 mg/ml.

[†] Lead concentration 0.06 μg/ml.

[§] Buffers made up as in Handbook of Biochemistry and Molecular Biology (3rd ed.).

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study--Cd, Pb, Hg, Pb and Zn. Forty-eight wells at a time were sampled by use of one rack containing the adsorption cartridges. Samples were withdrawn at 0, 0.5, 1, 3, 6, 9, 12, and 24 hours. The plates were kept in the dark at 22°C. Data from these experiments are shown as Figures 12 to 17.

Cadmium--Test species utilized in the cadmium uptake experiment were Navicula pelliculosa (at pH 6, 7 and 8) and Scenedesmus obliquus (at pH 6 and 7). Neither alga was found to accumulate cadmium, a finding inconsistent with earlier results. The control values for cadmium dropped inexplicably over the 24 hour period to the same extent as the experimental wells masking any uptake by either of the algae.

Lead--The results for the lead experiment are shown as Figures 12 through 14. For this experiment, the cultures were 15-day cultures; Chlamydomonas had a dry weight of 0.35 mg/ml while Nostoc 586 produced 0.27 mg/ml of dry weight. Once again, the efficiency of Chlamydomonas in removing lead is striking, with initial removal being most rapid at pH 6, where after only 30 minutes, experimental value was less than 50% of the control value. After 24 hours, experimental values at all pHs had risen to about 70% of the control values, perhaps as a result of a slow leaching of lead-organic complexes from the cell surface. Nostoc 586 was somewhat less efficient at removing lead in this experiment.

Mercury--The dramatic and rapid removal of mercury by the blue-green Nostoc H (15-day culture, 0.36 mg/ml dry weight) and Nostoc 586 (15-day culture, 0.27 mg/ml dry weight) was once again noted and is shown in Figures 15 to 17. A slow downward trend of control values at every pH may be explained by physical adsorption of the mercury on the plastic surfaces of the individual wells. Final experimental values were less than 20% of the control value at pH 6 for both strains of Nostoc at pH 6.

Zinc--No uptake was noted for zinc, a finding that was consistent with earlier results. Test species were Nostoc 586 (at pH 6 and 7) and Chlamydomonas at pH 6, 7, and 8).

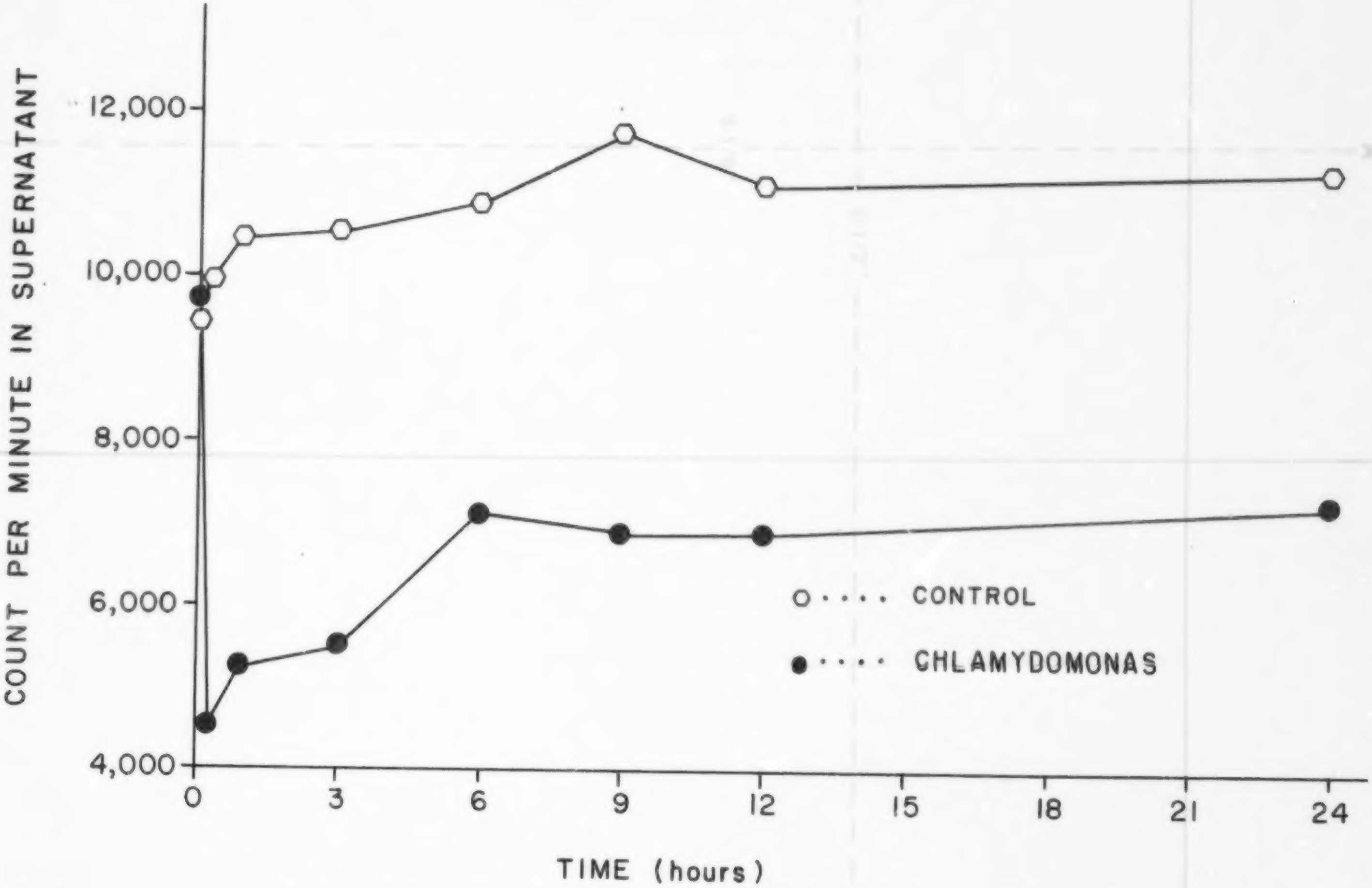

Figure 12. Uptake of lead - 210 at pH 6.

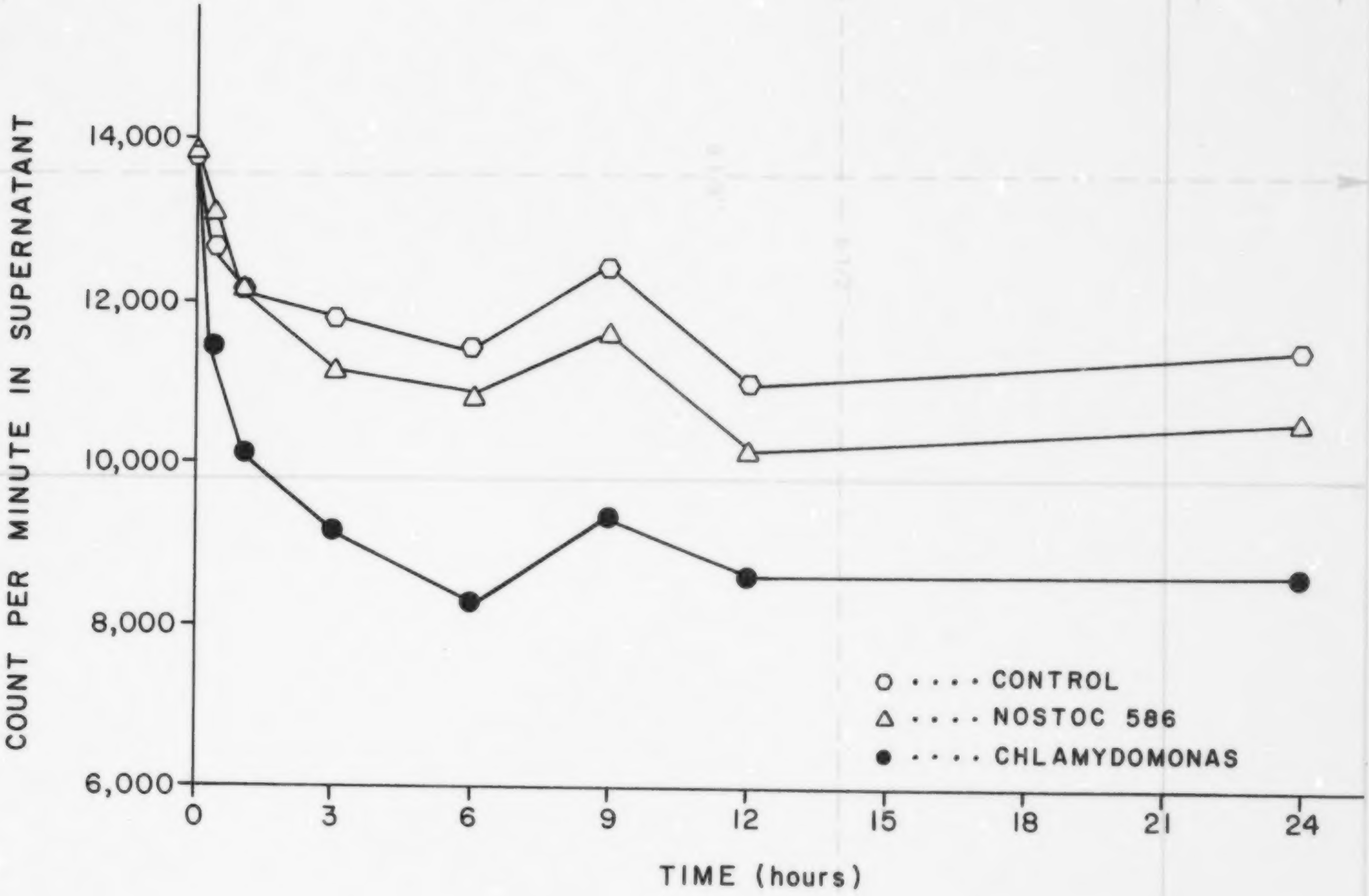

Figure 13. Uptake of lead - 210 at pH 7.

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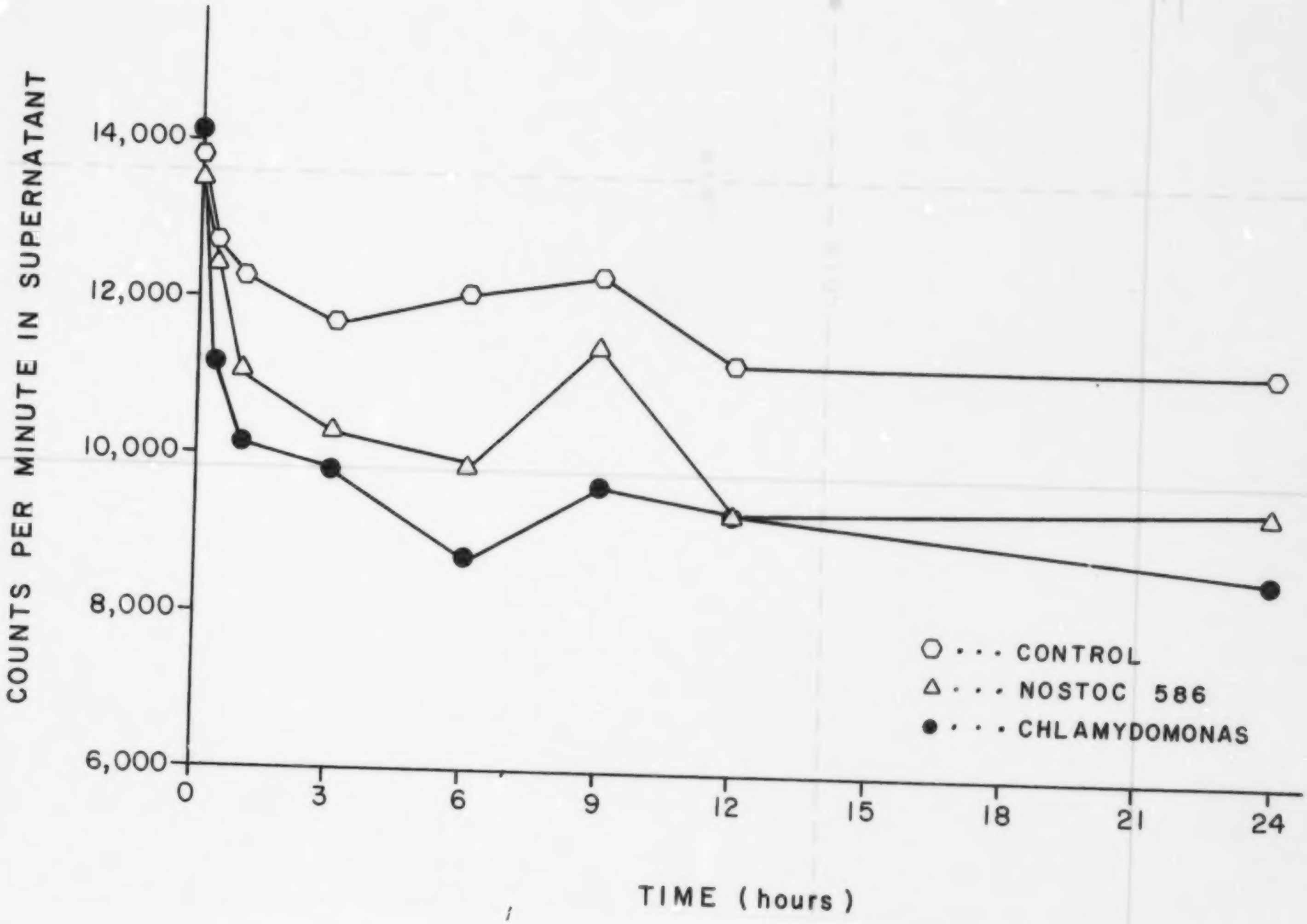

Figure 14. Uptake of lead - 210 at pH 8.

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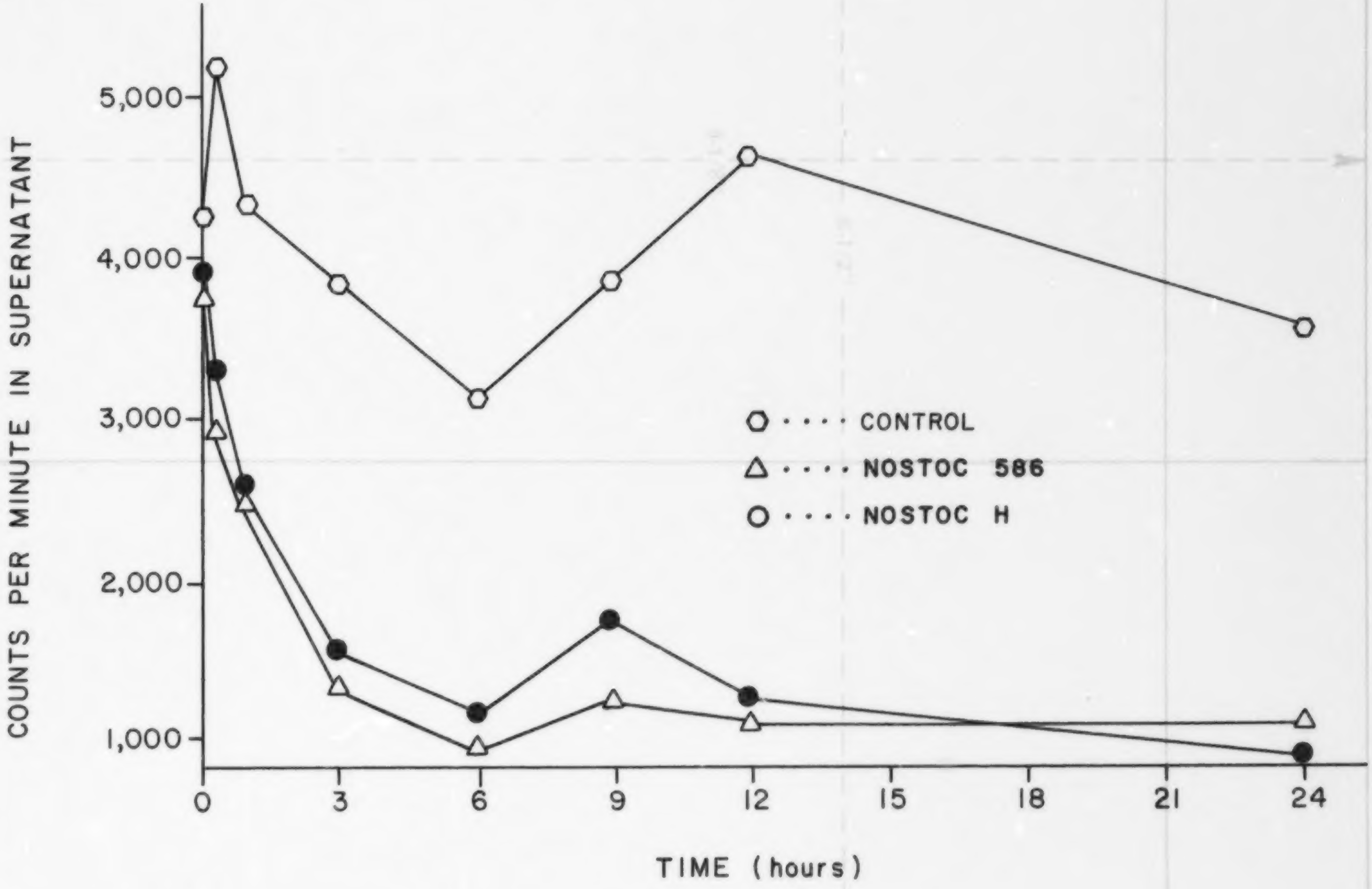

Figure 15. Uptake of mercury - 203 at pH 5.

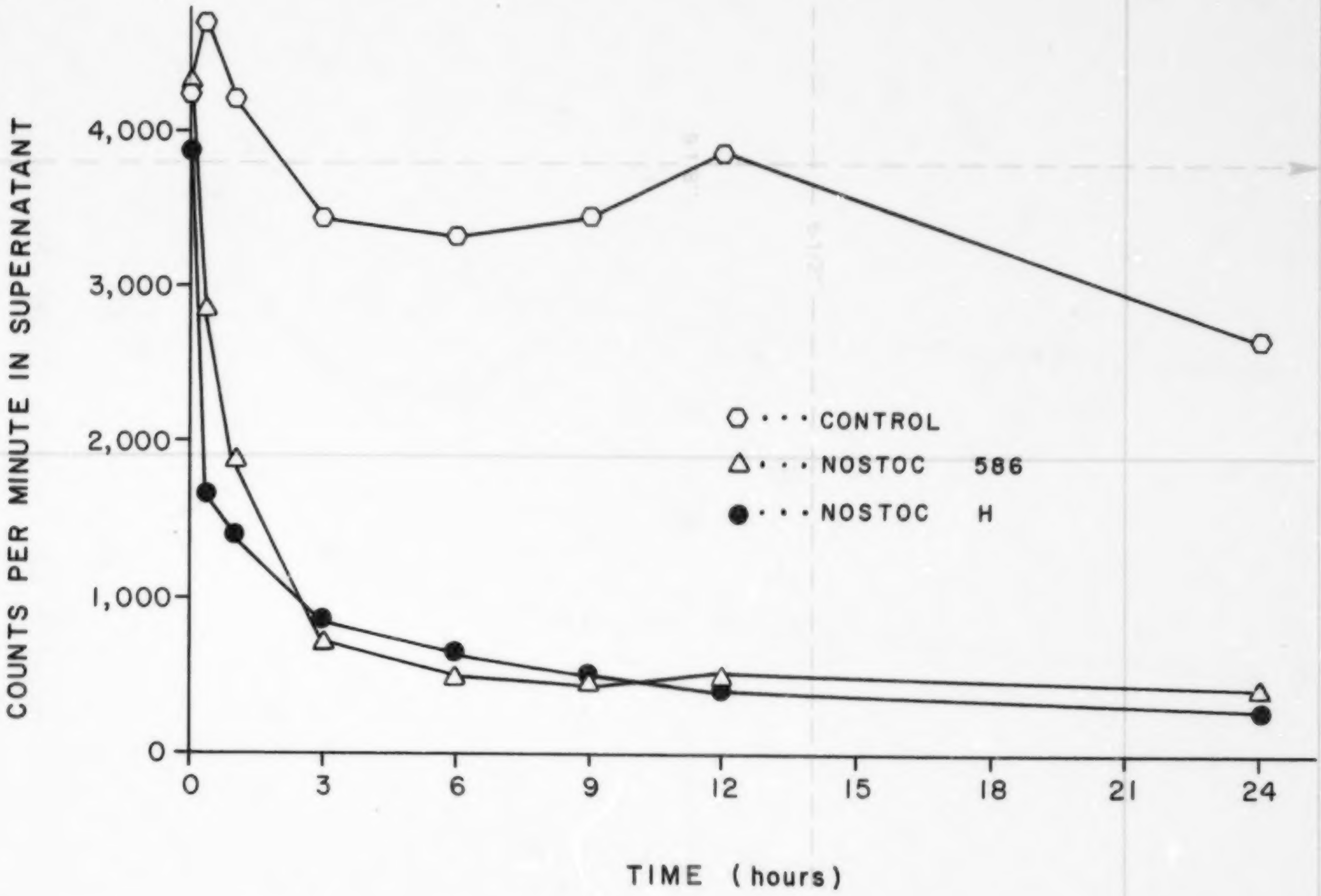

Figure 16. Uptake of mercury - 203 at pH 6.

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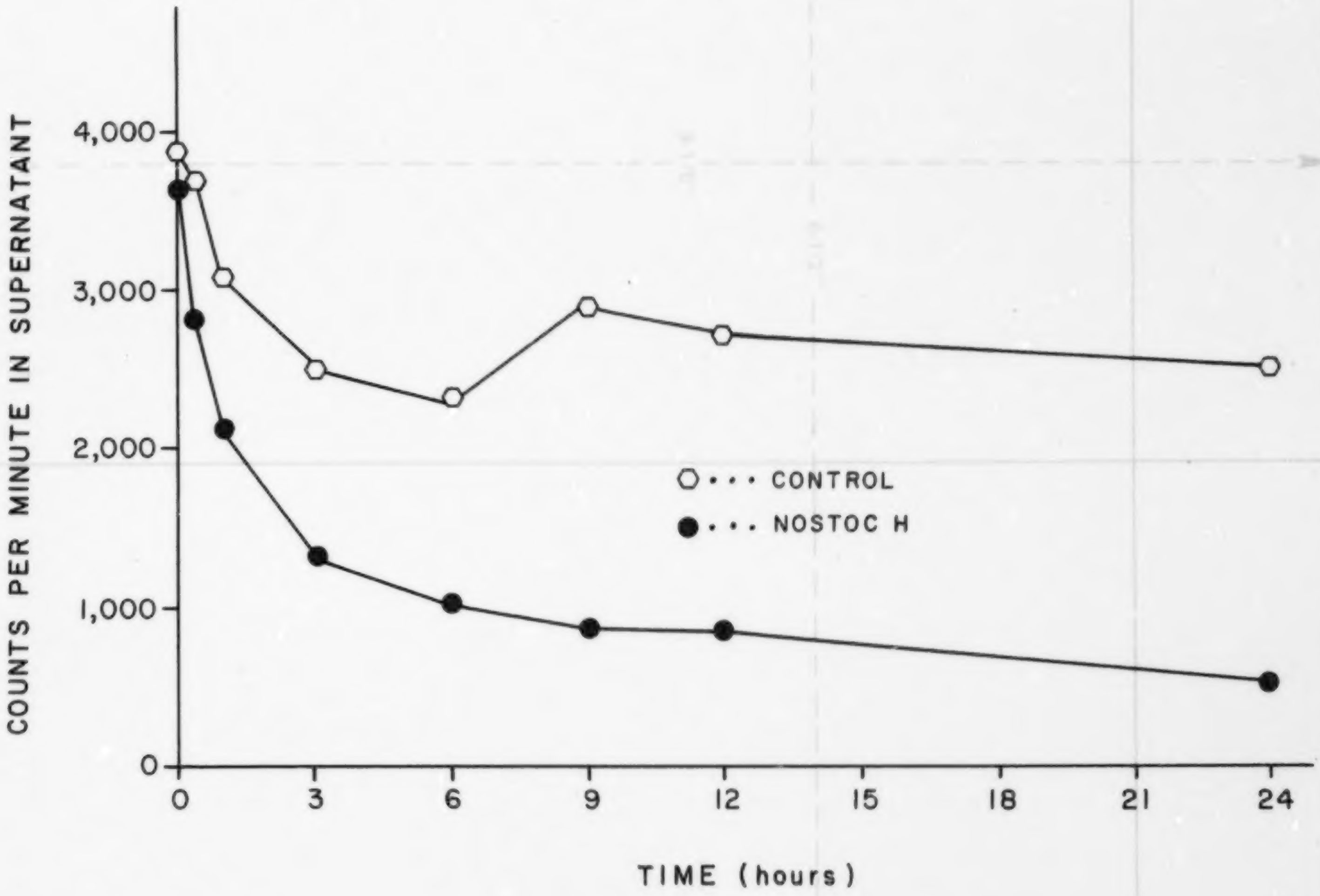

Figure 17. Uptake of mercury - 203 at pH 7.

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SECTION 7

DISCUSSION OF PROJECT RESULTS

The results of previous work, summarized in Section 4, were organized using the concentration factor (CF) as the means by which the ability of various algae to accumulate heavy metals was evaluated. Since this parameter was calculated for the algae used in the second phase of this study, a comparison seems appropriate and is provided in Tables 29 through 31.

Several comments about the data in these tables can be made. First published works by several investigators have shown zinc to be accumulated by a wide variety of algae, while this study has not yet found zinc to be amassed to any significant degree. However, previously cited work by Broda et al. (1971) indicated that zinc uptake by a species of *Chlorella* was light dependent. Since the present study represents an initial screening effort in which all cultures were kept in the dark, it is possible that this arbitrary selection of initial conditions prevented zinc uptake by the test algae employed in this study. An alternative hypothesis is that test algae did not accumulate zinc under any conditions.

With the exception of zinc, our results show reasonably good agreement with those reported in the literature, with all concentration factors between studies being within a factor of 2 to 3. As Copeland and Ayer (1972) point out, a concentration factor cannot be considered a fixed number, but is rather a variable dependent on environmental conditions. The agreement between these and previous studies seems reasonable.

Previous studies that have also examined the kinetics of heavy metal accumulation by algae, in general, report that uptake was an extremely rapid phenomenon (Schulze, Baldes and Lewin, 1976; Richardson, Millington and Miles, 1975; Kempner and Miller, 1972). The results of the 24 hour experiments in this study clearly confirmed the earlier observations in this regard. The negative results for the cadmium experiments cannot be explained at this time.

One obvious manner in which the present study extends previous work is the calculation of concentration factors for species of algae previously unstudied. These data (Tables 29, 30, & 31) were obtained under identical conditions and record both intraspecific (in the case of *Nostoc*) and interspecific differences in heavy metal accumulation by algae. New data are provided on the effect of culture age and initial pH during the labelling period on the accumulation of heavy metals by algae (Tables 29, 30, & 31) and deserve some comment.

The present study about doubles the number of algae for which concentration factors for cadmium have been determined (Tables 14 & 15). This is not only

TABLE 29. CONCENTRATION FACTORS FOR CADMIUM DETERMINED DURING THIS STUDY

Organism	Type	Culture Age[1]	Concentration Factor[2]
Navicula pelliculosa	Diatom	Young Old	$3.6 - 4.9 \times 10^3$ 70 - 320
Chlamydomonas	Green, flagellated	Young	5.5×10^3
Chlorella	Green, colonial	Young	$1.7 - 3.2 \times 10^4$
Scenedesmus obliquus	Green, colonial	Young Old	$2.7 - 3.6 \times 10^3$ 40 - 110
Ulothrix fimbrinata	Green, filamentous	Young	$1.1 - 1.4 \times 10^4$
Zygnema	Green, filamentous	Young	8.6×10^3
Gleotrichia	Blue-green, filamentous	Old	70 - 160
Nostoc 586	Blue-green, colonial	Young Old	$1.1 - 1.5 \times 10^3$ 50 - 90
Oscillatoria	Blue-green, filamentous	Young	$1.2 - 1.3 \times 10^3$
Nostoc H	Blue-green, colonial	Old	40 130

Notes: 1. Young = 11 day; Old = 44 day.

2. Concentration Factor = $\frac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

TABLE 30. CONCENTRATION FACTORS FOR LEAD AS DETERMINED DURING THIS STUDY

Organism	Type	Culture Age[1]	Concentration Factor[2]
Chlamydomonas	Green, flagellated	Young	$1.7 - 2.0 \times 10^4$
Mougeotia	Green, filamentous	Old Young	$3.0 - 3.6 \times 10^2$ 8.2×10^3
Ulothrix fimbrinata	Green, filamentous	Young	2.0×10^4
Nostoc 586	Blue-green, colonial	Young Old	$1.6 - 2.4 \times 10^3$ $1.4 \times 1.5 \times 10^2$
Nostoc muscorum A	Blue-green, colonial	Young	4.4×10^4
Nostoc H	Blue-green, colonial	Young Old	$1.5 - 1.6 \times 10^3$ $1.4 - 2.2 \times 10^2$
Nostoc L	Blue-green, colonial	Old	50 - 60
Nostoc W	Blue-green, colonial	Old	$1.2 - 1.5 \times 10^2$
Oscillatoria	Blue-green, filamentous	Young Old	1.5×10^3 90 - 260
Schizothrix calcicola	Blue-green, colonial	Young Old	$1.3 - 1.7 \times 10^3$ 60 - 90

Notes: 1. Young = 11 day; Old = 44 day.

2. Concentration Factor = $\dfrac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

TABLE 31. CONCENTRATION FACTORS FOR MERCURY AS DETERMINED DURING THIS STUDY

Organism	Type	Culture Age[1]	Concentration Factor[2]
Navicula pelliculosa	Diatom	Young	$3.5 - 6.8 \times 10^3$
Chlamydomonas	Green, flagellated	Young	$9.1 \times 10^3 - 1.6 \times 10^4$
Chlorella	Green, unicellular	Young	$2.4 - 4.7 \times 10^4$
Mougeotia	Green, filamentous	Young	$1.1 - 1.3 \times 10^4$
Scenedesmus obliquus	Green, colonial	Young Old	$3.5 - 6.5 \times 10^3$ $2.5 - 3.5 \times 10^2$
Ulothrix fimbrinata	Green, filamentous	Young	$1.8 - 2.6 \times 10^4$
Zygnema	Green, filamentous	Young	$9.2 \times 10^3 - 1.7 \times 10^4$
Gleotrichia	Blue-green, colonial	Old	$1.4 - 4.4 \times 10^2$
Nostoc 586	Blue-green, colonial	Young Old	$1.9 - 5.1 \times 10^3$ $1.4 - 3.6 \times 10^2$
Nostoc muscorum A	Blue-green, colonial	Young	$4.4 - 8.8 \times 10^4$
Nostoc H	Blue-green, colonial	Young Old	$1.6 - 3.3 \times 10^3$ $1.5 - 4.1 \times 10^2$
Oscillatoria	Blue-green, filamentous	Young	$2.3 - 3.4 \times 10^3$
Schizothrix calcicola	Blue-green, colonial	Young Old	$2.2 - 3.7 \times 10^3$ $2.9 - 3.7 \times 10^2$

Notes: 1. Young = 11 day old; Old = 44 day.

2. Concentration Factor = $\dfrac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

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important to waste treatment applications, but also to environmental impact studies. Also noteworthy is the close agreement of concentration factors from the literature with those of this study, with CFs on the order of 10^3 common to both. What is not immediately apparent is the generally lower CFs for the blue-green algae. In fact, three 10-day old cultures of blue-green algae, *Nostoc muscorum* A, *Nostoc* H and *Schizothrix calcicola* did not remove cadmium significantly at any pH, while only one green algae, *Mougeotia*, showed such negative results. The absence of blue-green algae in Table 29 leads one to wonder whether they were examined by other workers and found not to accumulate cadmium or whether the present study represents the first effort to study the uptake of cadmium by blue-green algae. Although the total number of species examined is small, the present study suggests that green algae are somewhat more efficient at accumulating cadmium than the blue-green algae.

Concentration factors for lead are generally on the order of 10^3 to 10^4 for all algae studied. All the blue-green algae screened were successful at removing lead at least at one pH while the diatom *Navicula pelliculosa* and the green *Chlorella* and *Scenedesmus obliquus* were not successful at accumulating lead at any pH. As will be discussed below, *Chlamydomonas* proved to be the most efficient at removing lead from solution.

Mercury was demonstrated again (Tables 18 and 19) to be concentrated avidly by most species of algae. Every organism screened in this study removed mercury at least at one pH, with concentration factors on the order of 10^3 to 10^4. Algae may be one of the most effective means for removing this element. Ben-Bassat and Mayer (1975) and DeFillipis and Pallaghy (1976) found that algae can reduce mercuric ion to elemental mercury which is subsequently volatilized to the atmosphere. One might well ask whether the same phenomenon occurred in our experiments, giving artificially high concentration factors. Although the possibility cannot be denied categorically, the short time scale of our experiments (3 hours) and the quiescent conditions under which the plates were held would argue against loss of very much mercury to the atmosphere. This is especially true when one recalls that most other studies employed aerated cultures and a time scale of several days. Ben-Bassat and Mayer (1977) also indicated that the reducing factor(s) were extracellular. Since the algal cells in the present study were washed and resuspended in distilled water before introduction to the assay plates, the concentration of any reducing factor(s) would be diminished, at least initially. However, the phenomenon of mercury reduction and volatilization by algae clearly deserves further study.

Another contribution of this work was the systematic study of the effect of pH on heavy metal accumulation. The studies cited from the literature were generally field observations during which the pH of the surroundings varied naturally, or were laboratory studies where the culture media characteristically determined the pH of metal uptake, and therefore, uptake across a wide variety of pH conditions could not be determined. In general, those algae which are most proficient at removing metals will remove them over a wide pH range. For example, *Chlamydomonas* removes lead across a pH range from 4 to 9 at about the same efficiency (0.69-0.80) while the less effective alga *Mougeotia* removes lead only at pH 9 and then only at an efficiency of 0.29. Clearly *Chlamydomonas* is more competent at removing lead from solution.

Other observations made during this study tend to confirm and extend suggestions made by earlier workers. For example, Hessler (1974) hypothesized that the flagella of *Platymonas subcordiformis* provided the sites for a significant fraction of the lead taken up by this algae. While this hypothesis was not specifically tested in the laboratory, the dramatic superiority shown by *Chlamydomonas* in removing lead, as compared with the other algae evaluated combined with the fact that *Chlamydomonas* was the only flagellated form used, argues for the importance of the flagella sites of lead accumulation.

Kerfoot and Jacobs (1976) noted that cadmium was accumulated to a greater extent by a green playtomonad than by a mixture of diatoms. As noted above, the green algae (especially *Chlorella*) screened in this study seemed to be more efficient accumulators of cadmium than either the diatom (*Navicula pelliculosa*) or the blue-green algae employed.

Perhaps one of the most important contributions of this work, however, was the adaption and development of microtiter equipment, especially the Titertek™ supernatant collection apparatus, to the study of the problem of algal uptake of heavy metals. The technique and procedures developed during the course of this study have already provided usable data on a relatively limited number of variables. Future work will no doubt utilize the rapidity and sensitivity of the technique to accumulate more data covering a wider range of variables.

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APPENDIX

Tables of Concentration Factors Arranged by Metal

TABLE A-1. ARSENIC CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type of Observation	Concentration Factor	Basis	References
Benthic algae	Assorted	Marine	Radioecological	$2x10^3$	Wet	Lowman, et al.
Brown algae	"	"	Ecological	$2x10^2$ $-6x10^3$	"	Bowen 1966
Phytoplankton	"	Lake Michigan	"	$1.5x10^3$	"	Copeland and Ayers 1972

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TABLE A-2 CADMIUM CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type of Observation	Concentration Factor	Basis	Reference
Benthic algae	Assorted	Marine	Radioecological	$2x10^2$	Wet	Lowman, et al.1971
Plankton	"	"	Ecological	$9.1x10^2$	"	Bowen 1966
Brown algae	"	"	"	$8.9x10^2$	"	"
Fucus vesiculosis	Brown, macroscopic	U.K. Coast	"	$2.7x10^3$	Dry	Preston, et al. 1972
Porphyra umbilicalis	Red macroscopic	"	"	$6.6x10^2$	"	"
Prasinecladus subsala	Green, flagellated	Lab culture (marine)	Stable isotope	$6.7x10^3$	"	Kerfoot and Jacobs 1976
Phaeodactylum tricornutum and Chaetoceras sp.	Diatoms	"	"	$4x10^3$	"	"
Cladophora fracta	Green, filamentous	Fresh Water	Radioecological	$1.6x10^4$	"	Polikarpov 1966
C. glomerata	"	"	"	$1.7x10^4$	"	"
Mougeotia sp.	"	"	"	$2.8x10^3$		"
Spirogyra crassa	"	"	"	$3.3x10^3$	"	"

TABLE A-3 COPPER CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type of Observation	Concentration factor	Basis	Reference
Phytoplankton	Assorted	Lake Michigan	Ecological	$1.2x10^3$	Dry	Copeland & Ayers 1972
Phytoplankton	"	Monterey Bay	"	$2.8x10^3$	Dry	Martin & Knauer 1972
Benthic algae	"	Marine	Radioecological	$1x10^2$	Wet	Lowman, et al. 1971
Phytoplankton	"	"	"	$3x10^4$	"	"
Plankton	"	"	Ecological	$1.7x10^4$	"	Bowen 1966
Brown algae	"	"	"	$1.0-9.2x10^4$	"	"
Fucus vesiculosis	Brown, macroscopic	U.K. Coast	" "	$2.5-2.7x10^4$	Dry	Bryan & Hummerstone 1975
Fucus vesiculosis	"	"	"	$4.5.x10^3$	"	Preston, et al. 1972
Porphyra umbilicalis	Red, macroscopic	"	"	$6.3x10^3$	"	"
Fucus vesiculosis	Brown, macroscopic	Menai Straights,U.K.	"	$2.5-6.4x10^3$	"	Foster 1976
Ascophyllum nodosum	"	"	"	$8.6x10^3$	"	"
Chondrus yendoi	Red, macroscopic	Sea of Japan	"	$2.3x10^3$	"	Saenko, et al. 1976

(continued)

TABLE A-3.(continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Ptilota filicina	Red, macroscopic	Sea of Japan	Ecological	5.2×10^3	Dry	Saenko, et al. 1976
Polysiphonia japonica	"	"	"	2.8×10^3	"	"
Rhodomela latrix	"	"	"	1.8×10^3	"	"
Heterochordaria abietina	Brown, macroscopic	"	"	8×10^2	"	"
Chorda filum	"	"	"	5.6×10^2	"	"
Laminaria chichoriodes	"	"	"	1.6×10^3	"	"
L. japonica	"	"	"	3.6×10^2	"	"
Agarum cribrosum	"	"	"	9×10^2	"	"
Pelvetia wrightii	"	"	"	4.4×10^2	"	"
Sargassum pallidum	"	"	"	1.7×10^3	"	"
Enteromorpha prolifera	Green, macroscopic	"	"	2.8×10^3	"	"
Ulva fenestrata	"	"	"	8.4×10^3	"	"

(continued)

TABLE A-3.(continued)

Organism	Type	Habitat	Type of	Concentration	Basis	Reference
Ulvaria splendens	Green, macroscopic	Sea of Japan	Ecological	$5.7x10^3$	Dry	Saenko, et al. 1976
Codium yessoensis	"	"	"	$5x10^2$	"	"
Chlamydomonas nivalis	Green, flagellated	Greenland snow	"	25	"	Fjerdingstad 1973
Mougeotia	Green, filamentous	Polluted Stream	"	$1.2x10^4$	"	Trollope & Evans 1976
Zygnema	"	"	"	$3.5x10^4$	"	"
Ulothrix	"	"	"	$1.0x10^4$	"	"
Cladophora	"	"	"	$1.8-3.5x10^4$	"	"
Spirogyra	Green, filamentous	Polluted stream	"	$3.3x10^3-1.4x10^4$	"	"
Oedogonium	"	"	"	$6.6x10^3$	"	"
Tribonemia	Yellow-green, filamentous	Polluted	"	$1.3-8.3x10^4$	"	"
Coccomyxa	Green, colonial	"	"	$5x10^4$	"	"
Oscillatoria	Blue-green filamentous	"	"	2.10^4	"	"

(continued)

TABLE A-3. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Scenedesmus[*]	Green, colonial	Lab culture	Stable isotope	$3.7\text{-}4.0\text{x}10^3$	Dry	Stokes 1975
Scenedesmus[†]	"	"	"	$2.5\text{-}3.0\text{x}10^3$	"	"
Scenedesmus[††]	"	"	"	$7.6\text{x}10^3\text{-}4.1\text{x}10^4$	"	"

[*] Laboratory strain.

[†] Strain isolated from metal polluted lake.

[††] Low value in absence of nickel: high value in presence of 3.0 pp. Ni.

TABLE A-4. LEAD CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Benthic algae	Assorted	Marine	Radioecological	$7x10^2$	wet	Lowman, et al. 1971
Phytoplankton	"	"	"	$4x10^3$	"	"
Plankton	"	"	Ecological	$4.1x10^4$	"	Bowen 1966
Brown algae	"	"	"	$7x10^4$	"	"
Fucus vesiculosa	Brown, macroscopic	U.K. Coast	"	$2.4x10^3$	Dry	Preston, et al. 1972
Porphyra umbilicalis	Red, macroscopic	"	"	2.10^3	"	"
Phaeodactylum tricornutum	Diatom	Lab culture (marine)	Stable isotope	$2x10^3$	"	Schulz-Baldes & Lewin 1976
Platymonas subcordiformis	Green, flagellated	"	"	$1x10^3$	"	"
Mougeotia sp	Green, filamentous	Polluted	Ecological	$2x10^4$	"	Trollope and Evans 1976
Tribonema	Yellow-green, filamentous	"	"	$3.0x10^3$-$1.8x10^4$	"	"
Coccomyxa	Green, colonial	"	"	$3.1x10^4$	"	"
Zygnema	Green, filamentous	"	"	$2.5x10^4$	"	"

(continued)

TABLE A-4. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Ulothrix	Green, filamentous	Polluted stream	Ecological	$7.6\text{x}10^3$	Dry	Trollope and Evans 1976
Oscillatoria	Blue-green filamentous	"	"	$5.6\text{x}10^3$	"	"
Microspora	Green, filamentous	"	"	$7.4\text{x}10^2$	"	"
Cladophora	"	"	"	$2.2\text{-}9.0\text{x}10^2$	"	"
Spirogyra	"	"	"	$4.0\text{x}10^2\text{-}3.8\text{x}10^3$	"	"
Oedogonium	"	"	"	$5.7\text{x}10^2$	"	"

TABLE A-5. MERCURY CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type Observation	Concentration factor	Basis	Reference
Phytoplankton	Assorted	Lake Michigan	Ecological	5.9×10^3	Wet	Copeland and Ayers, 1972
Phytoplankton	"	Monterey Bay	"	2.2×10^2	"	Knauer and Martin, 1972
Brown algae	"	Marine	"	2.5×10^2	"	Bowen, 1966
Cladophora fracta	Green, filamentous	Fresh water	Radioecological	5.9×10^3	Dry	Polikarpov, 1966
C. glomerata	"	"	"	5.4×10^3	"	"
C. glomerata[*]	"	Lab culture	Radioisotope	$6.5\times10^1-5.6\times10^3$	"	Burkett, 1975
Pediastrum boryanum	Green, colonial	"	"	1.8×10^4	Wet	Richardson et al., 1975
Synedra ulna[+]	Diatom	"	"	$1.3-1.7\times10^4$	"	Fujita and Hashizume,1975
Chlorella[+]	Green, unicellular	"	"	$7.10^1\times4\times8\times10^2$	"	DeFilippis and Pallaghy,1976a
Chlorella[††]	"	"	"	$1.6\times10^2-1.2\times10^3$	"	"
Phaeodactylum tricornutum	Diatom	Lab culture (marine)	"	1.3×10^4	"	Hannan, et al. 1973

[*] $CH_3{}^{203}HgCl$ [+] $^{203}HgCl_2$ [††] Phenyl ^{203}Hg acetate.

TABLE A-6. NICKEL CONCENTRATION BY ALGAE

Organism	Type	Habitat	Type observation	Concentration factor	Basis	Reference
Benthic algae	Assorted	Marine	Radioecological	$1x10^3$	Wet	Lowman,et al. 1971
Phytoplankton	"	"	"	$5x10^3$	"	"
Plankton	"	"	Ecological	$1.7x10^3$	"	Bowen, 1966
Brown algae	"	"	"	$1.4-5.0x10^2$	"	"
Phytoplankton	"	Monterey Bay	"	$5.7x10^2$	Dry	Martin and Knauer,1972
Fucus vesiculosis	Brown, macroscopic	U.K. Coast	"	$2.8x10^3$	"	Preston, et al., 1972
Porphyra umbilicalis	Red, macroscopic	"	"	$1.1x10^3$	"	"
Ascophyllum nodosum	Brown, macroscopic	Menai U.K.	"	$4.6x10^3$	"	Foster, 1976
Fucus vesiculosis	"	"	"	$2.8-6.8x10^3$	"	"
Chondrus yendoi	Red, macroscopic	Sea of Japan	"	20	20	Saenko, et al., 1976
Ptilota filicina	"	"	"	$2x10^2$	"	"
Rhodomela larix	"	"	"	$3x5x10^2$	"	"
Heterochordaria abietina	Brown, macroscopic	"	"	40	"	"

(continued)

TABLE A-6. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Chorda filum	Brown, macroscopic	Sea of Japan	Ecological	50	Dry	Saenko et al. 1976
Laminaria chichorioides	"	"	"	60	"	"
L. japonica	"	"	"	$3.2x10^2$	"	"
Agarum cribrosum	"	"	"	$3.2x10^2$	"	"
Pelvetia wrightii	"	"	"	$4.4x10^2$	"	"
Sargassum pallidum	"	"	"	$1.7x10^3$	"	"
Enteromorpha prolifera	Green, macroscopic	"	"	30	"	"
Ulva fenestrata	"	"	"	$2x10^2$	"	"
Ulvaria splendens	"	"	"	$7.8x10^2$	"	"
Codium yessoensis	"	"	"	30	"	"
Chlamydomonas nivalis	Green, flagellated	Greenland snow	"	$1.4x10^2$	"	Fjerdingstad 1973

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TABLE A-6. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Mougeotia	Green, filamentous	Polluted stream	Ecological	$1.6x10^3$	Dry	Trollope and Evans 1976
Zygnema	"	"	"	$6x10^3$	"	"
Ulothrix	"	"	"	$1.4x10^2$	"	"
Cladophora	"	"	"	$3.9-8.9x10^2$	"	"
Spirogyra	"	"	"	$2.5x10^2-1.7x10^3$	"	"
Oedogonium	"	"	"	$1.2x10^3$	"	"
Tribonema	Yellow-green filamentous	"	"	$1.1-3.0x10^3$	"	"
Coccomyxa	Green, colonial	"	"	$1.2x10^3$	"	"
Oscillatoria	Blue-green filamentous	"	"	$9.1x10^3$	"	"
Microspora	Green, filamentous	"	"	36	"	"
*Scenedesmus**	Green, colonial	Lab culture	Stable isotope	$1.2x10^3-8.2x10^3$	"	Stokes 1975

* Low value in absence of copper, high value in presence of 1.0 ppm Cu.

TABLE A-7. CONCENTRATION OF ZINC BY ALGAE

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Benthic algae	Assorted	Marine	Radioecological	4.1×10^2	Wet	Lowman, et al. 1971
Phytoplankton	"	"	"	1.5×10^4	"	"
Plankton	"	"	Ecological	$10^3 - 6.5 \times 10^4$	"	Bowen 1966
Brown algae	"	"	"	$10^2 - 1.3 \times 10^4$	"	"
Phytoplankton	"	Monterey Bay	"	5.5×10^3	Dry	Martin and Knauer 1972
Fucus vesiculosis	Brown, macroscopic	Marine	Radioecological	4.2×10^2	Wet	Polikarpov 1966
F. serratus	"	"	"	$6 \times 10^2 - 1.1 \times 10^3$	"	"
Ascophyllum nodosum	"	"	"	1.4×10^2	"	"
Pelvetia canaliculata	"	"	"	1×10^3	"	"
Laminaria saccharina	"	"	"	4.2×10^2	"	"
L. digitata	"	"	"	$4 \times 10^2 - 1 \times 10^3$	"	"
Ascophyllum nodosum	"	Norway Fjord	Ecological	50	"	Skipnes, et al. 1975
Fucus	"	Marine	Radioecological	1.1×10^3	"	Rice 1961

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TABLE A-7. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Pelvetia canaliculata	Brown, macroscopic	Marine	Radioecological	$1x10^3$	Wet	Rice 1961
Ascophyllum nodosum	"	"	"	$1.4x10^3$	"	"
Fucus vesiculosis	"	U.K. Coast	Ecological	$1.1-6.4x10^4$	Dry	Bryan and Hummerstove 1973
"	"	"	"	$2x10^4$	"	Preston, et al. 1972
"	"	Menai Straights U.K.	"	$1.0-6.4x10^4$	"	Foster
Ascophyllum nodosum	"	"	"	$1.3x10^4$	"	"
Fucus vesiculosis	Brown macroscopic	Lab culture (marine)	Radioisotope	$3.3x10^3$	Wet	Gutknecht 1965
Dictyota dichotoma	Golden macroscopic	"	"	$2.8x10^2$	"	"
Porphyra umbilicalis	Red macroscopic	"	"	$2.6x10^2$	"	"
Cystoseira barbata	Brown macroscopic	Marine	Radioecological	$1.9x10^2$	"	Polikarpov 1966
Heterochordaria abietina	"	Sea of Japan	Ecological	$1.5x10^2$	"	Saenko, et al 1976

(continued)

TABLE A-7. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Chorda filum	Brown, macroscopic	Sea of Japan	Ecological	60	Wet	Saenko, et al 1976
Laminaria chichorioides	"	"	"	$1.5x10^2$	"	"
L. japonica	"	"	"	70	"	"
Agarum cribrosum	"	"	"	2.10^2	"	"
Costaria costata	"	"	"	60	"	"
Pelvetia wrightii	"	"	"	20	"	"
Sargassum pallidum	"	"	"	40	Dry	"
Ulva nigida	Green, macroscopic	Marine	Radioecological	$1.3x10^2$	"	Polikarpov 1966
U. pertusa	"	"	"	2.9x10	"	"
U. fenestrata	"	Sea of Japan	Ecological	30	"	Saenko, et al. 1976
Ulvaria splendens	"	"	"	$1x10^2$	"	"
Codium yessoensis	"	"	"	$5.4x10^2$	"	"
Enteromorpha prolifera	"	"	"	$5x10^2$	"	"
Ulva lactua	"	Lab culture (marine)	Radioisotope	$2.9x10^2$	Wet	Gutknecht 1965
Codium decorticatum	"	"	"	30	"	"

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TABLE A-7. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Euglena viridis	Unicellular, euglenophyte	Lab culture	Stable isotope	$2.4x10^3$-$1.1x10^5$	Dry	Coleman, et al. 1971
Chlorella vulgaris	Green, unicellular	"	"	$4.2x10^3$-$9.6x10^4$	"	"
Scenedesmus quadricauda	Green, colonial	"	Radioisotope	$7.8x10^2$	"	Polikarpov 1966
Cladophora fracta	Green, filamentous	Fresh water	"	$6.1x10^3$	"	"
C. glomerata	"	"	"	$3.9x10^3$	"	"
Mougeotia	"	"	"	$3.7x10^4$	"	"
Spirogyra crassa	"	"	"	$1.9x10^3$	"	"
Chlorella	Green, unicellular	"	"	$1.4x10^2$	"	"
Scenedesmus quadricauda	Green, colonial	Lab culture	"	$7.8x10^2$	"	Gileva 1960
Cladophora glomerata	Green, filamentous	"	"	$3.9x10^3$	"	"
Spirogyra	"	"	"	$3.2x10^4$	"	"
"	"	Polluted stream	Ecological	6.6-$9.0x10^3$	"	Trollope and Evans 1976

(continued)

TABLE A-7. (continued)

Organism	Type	Habitat	Type of observation	Concentration factor	Basis	Reference
Mougeotia	Green, filamentous	Polluted stream	Ecological	1.3×10^3	Dry	Trollope and Evans 1976
Zygnema	"	"	"	4.0×10^3	"	"
Ulothrix	"	"	"	7.3×10^2	"	"
Cladophora	"	"	"	$4.0 \times 10^3-1.2 \times 10^4$	"	"
Oedogonium	"	"	"	1.5×10^3	"	"
Tribonema	Yellow-green, filamentous	"	"	1.7×10^3	"	"
Coccomyxa	Green, colonial	"	"	1.7×10^2	"	"
Oscillatoria	Blue-green, filamentous	"	"	9.6×10^2	"	"
Spirogyra	Green, filamentous	Fresh water	Radioisotope	3.2×10^4	"	Pollikarpov 1966
Chara	"	"	"			
C. aspera	"	"	"	2.3×10^3	"	"
C. fragalis	"	"	"	2.9×10^3	"	"

* Values obtained from factorial experiment involving 8 temperatures and 6 salinities.

+ Minimal medium experiment.